Lifestyle Interventions in Women with PCOS:

The Role of a Pulse-Based Diet

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ABSTRACT

Context: Polycystic ovary syndrome (PCOS) is complex disorder associated with many metabolic abnormalities. PCOS is one of the most common endocrine disorders occurring in women of reproductive age and affects about 6-7% of the population. Women with PCOS have insulin resistance and hyperinsulinemia, thus increasing their risk of developing Type 2 diabetes mellitus, dyslipidemia, hypertension, cardiovascular disease, and endometrial cancer

Overall Objective: To compare anthropometric features (weight, BMI, WC, body fat percent), antral follicle count (AFC), fasting glucose and insulin levels, HOMA score, menstrual bleeding patterns, and abdominal adiposity before and after a dietary intervention.

Materials and Methods: The work presented herein represents a subset of the data being analyzed in an ongoing study titled "Lifestyle Intervention for Women with Polycystic Ovary Syndrome: The Role of a Pulse-Based Diet and Aerobic Exercise on Infertility Measures and Metabolic Syndrome Risk". PCOS was diagnosed by two of the three diagnostic criteria as defined by the Rotterdam consensus: a history of cycles >35 days in length, hyperandrogenism as defined by a Ferriman and Gallwey score of >6 or hyperandrogenemia, as well as polycystic ovaries (PCO), defined by >25 follicles visualized upon transvaginal ultrasonography (TVU). Participants were randomized to either a 16 week pulse-based diet or to a TLC diet for 16 weeks. All participants were asked to follow an exercise program for the 16 week duration of the intervention. Changes in demographic, anthropometric features AFC, fasting insulin levels, and intervals between menstrual cycles were assessed.

Results: Twenty four women completed the 16 week dietary intervention to date (pulse n=13, TLC n=11). Participants were found to be similarly matched for age, weight, BMI, WC, and

FAI. Weight (p=0.002) and body fat (p=0.0004) decreased significantly. No significant differences were detected in BMI and waist circumference. Antral follicle counts were decreased in the right ovary (p=0.04) but not the left ovary (p=0.11). There was no change in fasting glucose levels detected. There was a decrease in fasting insulin levels (p=0.02) and in HOMA score (p=0.02). No change in abdominal adiposity was detected (p=0.88). There was a tendency toward a change of fasting insulin levels and HOMA score due to the pulse-based diet. The average interval between menses decreased after the intervention (p=0.04). The longest length of time between menses also decreased after the intervention (p=0.01).

Conclusions: Our hypothesis was partially supported. We observed significant decreases in weight, body fat percent, AFC in the right ovary, fasting insulin levels and intermenstrual intervals. In most women, the decreased intermenstrual interval translated into the resumption of menstrual cyclicity. However, the participants' BMI, WC, AFC in the left ovary, and abdominal adiposity were not affected. Consuming food of a lower glycemic index without a calorie restriction may help women with PCOS gain healthier anthropometric profiles, decrease serum insulin levels and insulin resistance, and increase the regularity of menstrual cycles. Further study involving weight reduction and dietary intervention with pulses may prove to be more successful than calorie reduction alone.

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To my mom, my dad, my grandparents, sisters, aunts and uncles, I love you. Thank you for your support and love even from really far away.

DEDICATION

To Lilly and Francis D'Souza,

You are best grandparents one could ever have.
You have loved me like your own since I was born and
among the countless stories and walks and sneaky snacks,
it was your love in me that has kept me going.
I hope I make you as happy and proud as you make me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

17-Beta-Hydroxysteroid Dehydrogenase	17β-HSD
Abdominal Region Fat Quantity	R1
American Society for Reproduction	ASRM
Anti-Mullerian Hormone	AMH
Antral Follicle Count	AFC
Body Fat Distribution	BFD
Body Mass Index	BMI
Cardiovascular Disease	CVD
Computed Tomography	CT
Corpus Luteum	CL
C-Reactive Protein	CRP
Cyclic Adenosine Monophosphate	cAMP
Dehydroepiandrosterone	DHEA
Dihydrotestosterone	DHT
Dominant Follicle	DF
Dual X-Ray Absorptiometry	DEXA
Epidermal Growth Factor	EGF
European Society for Human Reproduction and Embryology	ESHRE
Follicle Stimulating Hormone	FSH
Food and Drug Administration	FDA
Free Androgen Index	FAI
Free Fatty Acids	FFA
Frequently Sampled IV Glucose Tolerance Test	FSIGT

Glucose Transporter 4	GLUT4
Glycemic Index	GI
Gonadotropin Releasing Hormone	GNRH
Granulosa Cells	GC
High Density Lipoprotein	HDL
Homeostasis Model Asssessment	HOMA
Impaired Glucose Tolerance	IGT
Insulin Receptor Substrate-1	IRS-1
Insulin Resistance	IR
Insulin-Like Growth Factor	IGF-1
Kit Ligand	KL
Low Density Lipoprotein	LDL
Luteinizing Hormone	LH
Maximal Aerobic Capacity	VO_2max
Metabolic Syndrome	MBS
National Institutes of Health	NIH
Non-Classical Adrenal Hyperplasia	NCAH
Normal Glucose Tolerance	NGT
Oral Glucose Tolerance Test	OGTT
Phosphatidylinositol 3-Kinase	PI3-K
Polycystic Ovaries	PCO
Polycystic Ovary Syndrome	PCOS
Randomized Control Trials	RCT
Sex Hormone Binding Globulin	SHBG
Structured Exercise Training	SET

Dehydroepiandrosterone-Sulfate	DHEA-S
Testosterone	T
Therapeutic Lifestyle Changes	TLC
Third National Health and Nutrition Examination Survey	NHANES III
Transvaginal Ultrasonography	TVU
Trigylcerides	TG
True Negative Rate	Specificity
True Positive Rate	Sensitivity
Tumor Growth Factor alpha	TGF-α
Type 2 Diabetes Mellitus	T2DM
Waist Circumference	WC
Waist Hip Ratio	WHR

CHAPTER 1

GENERAL INTRODUCTION

Polycystic ovarian syndrome (PCOS) is complex disorder associated with many metabolic abnormalities. A positive diagnosis is made if two of the following three criteria are met: 1) clinical and/or biochemical hyperandrogenism; 2) chronic anovulation; and, 3) polycystic ovaries as visualized ultrasonographically. PCOS is one of the most common endocrine disorders occurring in women of reproductive age and affects about 6% to 7% of the population (1). The prevalence of PCOS is approximately 6.5% to 8% when biochemical/clinical features are used for diagnosis and approximately 20% using ultrasonography. In Canada, approximately 1.4 million women may exhibit varying degrees of PCOS (2).

Fifty to 70% of women with PCOS have insulin resistance and hyperinsulinemia, thus increasing their risk of developing Type 2 diabetes mellitus, dyslipidemia, hypertension, cardiovascular disease, and endometrial cancer (3). Women with PCOS are four times as likely to develop Type 2 diabetes mellitus as compared with BMI matched controls.

There is no main treatment or drug to combat PCOS. Clinicians will often separate and treat the external signs of reproduction and not treat or ignore the metabolic complications associated with PCOS. The heterogeneity n the PCOS phenotype can also add to the complexity of treating PCOS. In the context of human diets and the present study, lifestyle interventions have been shown to have positive effects in improving clinical and metabolic parameters in women with PCOS. The effects include decreased insulin resistance, improvement in anthropometric features, and increased menstrual cycle regularity (4).

In the context of the present study, pulses refer to a group of more than sixty different grain legumes grown around the world that are classified as low-fat sources of proteins and carbohydrates. Examples of pulse crops include chickpeas, beans, peas, and lentils. Pulse consumption decreases the rate of carbohydrate absorption, effectively lowering the glycemic index (GI) of a diet. Low GI diets prevent the onset and impact of Type 2 diabetes mellitus (5).

The cost of treating PCOS exceeds $4 billion in the United States alone. Therefore, early intervention by family doctors or allied health professionals should be undertaken in order to alleviate many of the physiologic and health concerns in women with PCOS (6).

CHAPTER 2

LITERATURE REVIEW

2.1 Brief History of the Classification of PCOS

Polycystic ovary syndrome (PCOS) was first established by Stein and Leventhal in 1935 to describe women who presented with obesity, hirsutism, and chronic anovulation and enlarged ovaries postmortem (7). Their original characterization has been modified in subsequent years to include biochemical profiles and ovarian ultrasonographic morphology (8). In 1990, a National Institutes of Health (NIH) conference defined two criteria for the diagnosis of PCOS: evidence of hyperandrogenism and ovarian dysfunction (9). In 2003, the representative experts from the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproduction (ASRM) met in Rotterdam, Netherlands and amended the definition of PCOS to reflect a positive diagnosis if two of the following three criteria were met: 1) clinical and/or biochemical hyperandrogenism; 2) chronic anovulation; and, 3) polycystic ovaries as visualized ultrasonographically. None of the criteria were considered absolutely required for diagnosis of PCOS due to the heterogeneity in presentation. PCOS was classified as a disorder of exclusion; other causes of anovulation, androgen excess, or related disorders, such as congenital adrenal hyperplasia, hyperprolactinemia, thyroid disease, and hypothalamic suppression must be excluded before diagnosis (10).

2.2 Diagnostic Criteria of PCOS

2.2.1 Hyperandrogenemia

Hyperandrogenemia, defined as elevated levels of androgens in systemic circulation, is present in 60% to 80% of women who present with PCOS (11). Androgen excess appears to be central to the symptoms of PCOS (12). Hormonal profiles used to test for hyperandrogenism include measurement of total, unbound, or free forms of testosterone (T, nmol/L). Twenty percent to 30% of women with PCOS have adrenal androgen excess. The androgens secreted by the adrenal glands include dehydroepiandrosterone (DHEA) and its sulfate dehydroepiandrosterone (DHEA-S). Adrenal hyperandrogenism is a feature common to several diagnoses; non-classical adrenal hyperplasia (NCAH), adrenal androgen-producing tumors, and Cushing's syndrome. PCOS and NCAH may be clinically indistinguishable. Elevated levels of 17-hydroxyprogesterone can be used as a diagnostic factor to distinguish NCAH from PCOS. Adrenal androgen-producing tumors are identified by imaging studies and venous sampling. If Cushing's syndrome is suspected, measurement of 24 h urinary free cortisol or a low dose dexamethasone suppression test is typically performed to diagnose adrenal causes of hyperandrogenicity, polycystic ovaries and anovulation (13).

Clinical hyperandrogenism may be manifest phenotypically by the presence of hirsutism, acne, and/or alopecia (8). Hirsutism is defined as excessive terminal hair growth that follows a male pattern of distribution. The most common method used to score hirsutism is the Ferriman-Gallwey score. Nine body areas (upper lip, chin, chest, upper back, lower back, upper and lower abdomen, upper arm, and thigh) are assigned a score from 0 to 4 depending on the density of hair growth. The clinical evaluation of hirsutism is subjective and prone to inter-observer variability. Requisite threshold scores for hirsutism have varied from 6 to 8 (14). Is it important to note that the degree of hirsutism varies across ethnicities. There are racial variations in hair growth patterns with a lower prevalence in women of East Asian descent and a higher prevalence is seen in women of East Indian descent (8). Azziz et al. (8) observed that 659 of 1000 women who presented with androgen excess exhibited hirsutism. Approximately 65% to 75% of women with PCOS are affected by hirsutism.

Acne may be considered another sign of androgen excess. Acne affects approximately around 12% to 14% of Caucasian women with PCOS (15). Higher incidences of acne are observed in South Asian women (16). It is difficult to ascertain a causal relationship for acne in PCOS because of the prevalence of acne in the healthy population. There is also a lack of large scale studies of women with PCOS who present with acne but do not exhibit hirsutism (15).

Alopecia is the loss of hair from the head or the body and its presence has an unclear relationship with PCOS. The typical pattern of hair loss in women with PCOS involves thinning of the crown with preservation of the anterior hairline (17). It is difficult to separate alopecia as a symptom separate from hirsutism or as a symptom independently correlated with hyperandrogenism (15). Alopecia may be the sole dermatological sign of PCOS, but further studies are needed to confirm this hypothesis (17). While 75% to 80% of women who present with hirsutism will have PCOS and 20% to 40% of women who present with just acne have PCOS, only 10% of women who present with alopecia will have a positive PCOS diagnosis (15).

2.2.2 Anovulation

Women who ovulate regularly typically have regular menstrual cycles between 26 days to 35 days (18). Anovulation in women with PCOS results in oligo-amenorrhea which is less than 8 menstrual cycles per year or intervals of ≥ 35 days between menstrual cycles (11). A study of menstrual cyclicity in 873 women from the ages of 15 to 46 years revealed that 18% of the women had menstrual dysfunction and approximately 27% of the dysfunction group had PCOS as defined by the NIH criteria. It has been suggested that approximately 25% to 33% of women who present with menstrual dysfunction have PCOS (15).

2.2.3 Polycystic Ovaries

The morphology of polycystic ovaries (PCO) can be observed by ultrasonographic examination. Polycystic ovaries differ from “normal” ovaries by the distribution and number of ovarian follicles. Typically, follicles assume a characteristic peripheral ‘string of pearls’ distribution. The 2003 Rotterdam consensus defined PCO by the appearance of more than 12 follicles measuring 2 to 9 mm or a volume of >10mL (10). Recently, a higher threshold follicle number (more than 19 and 25 follicles) has been recommended to define the PCO. The higher threshold for follicle number obviates the growing misconception that polycystic ovaries have been highly prevalent in the general population of women who do not have PCOS (2).

2.3 Reproductive Function in Women

2.3.1 The Ovary and Its Structures

The primary roles of the human ovary are endocrine and exocrine functions. These include production of reproductive hormones, release of an oocyte that has the capacity to undergo fertilization and embryonic development, and ensuring the accessory reproductive organs are ready to support a pregnancy. The ovary consists of two zones, an outer cortex and the inner medulla. The cortex is covered by an outer layer of connective tissue called the tunica albuginea and an inner zone that contains follicles. A follicle is a fluid-filled sac that contains the oocyte. Primordial follicles are located in the avascular layer within the ovarian cortex beneath the tunica albuginea. Growing follicles and atretic follicles are found in the vascularized cortico-medullary border. The medulla of the ovary contains dense connective tissue with a network of stromal cells, blood vessels, and lymphatics (19).

2.3.2 The Menstrual Cycle

The menstrual cycle is controlled by endocrine, autocrine, and paracrine factors that regulate follicular recruitment and development, ovulation, luteinization, luteolysis, and endometrial growth (18). The menstrual cycle is traditionally divided into a follicular phase and a luteal phase. During the follicular phase, a cohort of follicles is recruited to grow and one is physiologically selected to become the dominant follicle (DF) under the influence of follicle stimulating hormone (FSH). In women, usually the DF emerges on day 3 of the cycle at a 6 mm diameter (20). Visual identification of the DF usually occurs at approximately10 mm in diameter 3 to 4 days later. The remaining cohort follicles undergo atresia. Women have major follicle waves that lead to DF selection in addition to minor waves which have follicle recruitment but no DF selection. The DF ovulates in response to the elevation of estradiol which leads to the release of a surge of luteinizing hormone (LH). The formation of a corpus luteum (CL) after ovulation establishes the start of the luteal phase (21). During the luteal phase, the CL secretes progesterone to prepare the uterine lining for implantation and to maintain an early pregnancy. In women, if an oocyte is not fertilized there is no hormonal signal from an embryo to the CL to maintain CL activity. The CL degenerates and the progesterone levels drop leading to the start of menses (20).

Menses (menstrual bleeding) is the external sign that a reproductive cycle has progressed from follicle growth to ovulation to CL regression. Menses occurs cyclically at the end of the luteal phase and at regular intervals in women who ovulate regularly and signals the beginning of the next follicular phase. Menses usually lasts for 3 to 6 days (18).

The average reproductive lifespan of a woman is approximately 36 years and is described as the time from menarche as early as 8.5 years of age to 13 years of age to menopause at approximately 51 years of age. The menstrual cycles may change becoming exceedingly irregular after 35 years of age as women grow older and the number of oocytes decrease (22, 23).

Tracking human reproductive cycles using only the observation of menses is challenging as ovulation is not an obvious physical event. In addition, observation of cyclic menses does not necessarily indicate that there is normal ovulation. Seven percent of women with menstrual cycles of normal length are anovulatory (22). A menstrual cycle (the interval from the first day of menses to the first day of the next menses) in young healthy women of proven fertility is on average 28 days: the follicular phase lasts approximately 14.6 days and the luteal phase lasts 13.6 days (24). However, menstrual cycle length is highly variable among women of a similar age (21 to 35 days) with wide ranges in the follicular (10 to 23 days) and luteal phase (7 to 19 days) (22, 25, 26). Most of the variability in cycle length is due to variability of the follicular phase (18).

There is a close relationship between follicle wave development in the ovary, DF selection, and the pattern of FSH release from the pituitary (27). In women with regular intervals between menstrual cycles (21 to 35 days), FSH increases about 4 days before menses at the luteal-follicular transition, peaks on the day of emergence of the DF, and then slowly declines until the LH surge preceding ovulation (28, 29). A rise in estrogen during the follicular phase begins after the day of emergence of the DF and rapidly rises after DF selection (29). Twenty-four to 36 hours after the estrogen peak, an LH surge occurs, which stimulates ovulation (23). Progesterone levels begin to rise slightly two days before the LH surge and ovulation. Following ovulation, progesterone is consistently secreted by the luteinized cells of the former follicle wall, which develops into a discreet transitory organ, the CL. The CL is stimulated by low levels of LH, and progesterone is produced. Progesterone levels peak 6 days post ovulation and decline thereafter if pregnancy is not established. Menses occurs after progesterone and estradiol levels decline (18).

Other hormones involved in the menstrual cycle include inhibin A, inhibin B, and activin A. The luteal-follicular transition phase is coupled with the fall in inhibin A, estrogen, and progesterone and a rise in activin A (29, 30). Secretion of estrogen and inhibin A by the dominant follicle bring about the decline in FSH during the follicular phase and luteal secretion of both inhibin A and B maintain low FSH following ovulation (31).

2.3.3 Folliculogenesis in Women with Regular Reproductive Function

The primordial follicle consists of an oocyte arrested at the diplotene stage of prophase one which is surrounded by squamous granulosa cells (GC). When growth is initiated in primordial follicles, the oocyte begins to synthesize RNA followed by enlargement of the squamous GC. The first stage is a single layer of mixed squamous and cuboidal GC (intermediate follicles) and later change into cuboidal cells (primary follicle). Once GC proliferation develops into two or more layers, a secondary follicle is formed. Theca interna cells are recruited from surrounding stromal stem cells and are organized into layers around the follicle and are responsible for mesenchymal-epithelial cell interactions that aid in development of the follicle and oocyte (32, 33).

Primordial follicle growth is controlled by autocrine and paracrine factors. Primordial follicle growth is only minimally dependent upon FSH because of a low concentration/number of FSH receptors but is dependent on paracrine/endocrine factors (33). Granulosa cell-derived paracrine factors can activate or inhibit primordial follicles (kit ligand KL, TGF-α, EGF) depending on the environment of secretion (34). Granulosa cell-derived KL and its receptor, c-kit, is important in initiation, mesenchymal-epithelial cell signaling, and developing the oocyte in early folliculogenesis**.** Kit ligand causes secondary follicles to acquire FSH, estrogen, and androgen receptors and to be coupled by gap junction to facilitate cellular interactions (33). Follicle recruitment ends with the formation of antral (tertiary) follicles. Antral follicles are characterized by slower oocyte growth, extracellular fluid which accumulates to form an antrum and differentiation of GC layers into mural and cumulus cell subpopulations. In humans, growth of hundreds of primordial follicles is initiated, but during each menstrual cycle only about 10 to 20 antral follicles (2 to 5 mm in size that respond to FSH) remain and one physiologically selected follicle proceeds to ovulation. The process from initiation of growth to follicle selection takes approximately 150 days. Only the final two weeks of follicular development are dependent on systemic cyclic gonadotropic changes (32, 33).

2.4 Physiological Differences in Reproductive Function in Women with PCOS

2.4.1 The Cause and Effect of Hyperandrogenism

In the late 1960s, Ryan and Petro first proposed the two cell/two gonadotropin theory. They proposed that thecal cells produced androgens which were converted to estrogens by aromatase present in GC (35). Theca cells are located in close proximity to the basement membrane surround the GC layer, and are the major source of follicular androgens (36). One mechanism by which hyperandrogenism may be manifest in women with PCOS is through hyperactivity of the thecal cell layer. Follicles in polycystic ovaries contain an increased number of steroidogenic cells in the thecal layer and a decreased number of GC compared with ovaries of healthy women (37). An increase in testosterone production by thecal cells has been shown to arise from an increase in the synthesis of testosterone precursors and is not a result of dysregulation of testosterone synthesis (38). Theca cells in women with PCOS have increased expression of steroidogenic enzymes involved in androgen biosynthesis, such as cytochrome P450, 17 alpha-hydroxylase/17-20-lyase (P450c17), and 3beta-hydroxysteroid dehydrogenase

(39). There is also an increase in 5α-reductase activity in GC and an increase in the conversion of androstenedione to 5α-androstane-3,17-dione, a competitive inhibitor of aromatase activity. In women with PCOS, there is a sufficiently elevated concentration of 5α-androstane-3, 17-dione in the follicular fluid to alter aromatase activity (40).

Sixty percent to 80% of women with PCOS have elevated circulating androgens (11). Reproductively active sex steroids, such as testosterone and dihydrotestosterone circulate bound to sex-hormone binding globulin (SHBG), a plasma glycoprotein, and other proteins (12, 41). Sex hormone binding globulin is synthesized in the liver and is regulated by several metabolic and hormonal factors such as insulin. Insulin has been found to down-regulate SHBG production (41). Down-regulation of SHBG results in an increase in free testosterone which can enter cells and alter cellular function (12). The free androgen index (FAI) is a ratio of total testosterone to SHBG levels in the blood [(total testosterone/SHBG)*100], which is used as a measure of androgen status (42). Approximately 70% of PCOS patients have increased free testosterone blood levels and subsequently a higher FAI. Unfortunately, many of the of the commonly available, less expensive assays used to measure testosterone are most effective for higher levels detected in men and are highly variable in women who have lower levels and therefore unreliable for assessing women with PCOS. It is difficult to ascertain a consistent, reliable standard for the normative testosterone range in circulation (12).

2.4.2 Menstrual Cycle Irregularity in Women with PCOS

Women with PCOS can have ovulatory cycles; however, most women with PCOS experience menstrual cycle irregularity due to anovulation (12). An ovulatory menstrual cycle is characterized by endometrial growth that is stimulated by estrogen produced by the dominant follicle (DF) during the follicular phase. After the DF ovulates, progesterone is produced by the

CL. Endometrial proliferation decreases under the influence of progesterone. If pregnancy does not occur, the CL undergoes atresia, progesterone decreases and menses occurs as a consequence of progesterone withdrawal. The decrease of progesterone precipitates menstruation where the entire endometrial lining is shed. Menses occurs cyclically in women who ovulate at regular intervals (18).

Women with PCOS who do not ovulate have irregular, non-cyclic bleeding patterns. Anovulatory bleeding arises from estrogen stimulated endometrial tissue that has not been exposed to progesterone. Androgens synthesized in ovarian stromal tissue are aromatized to estrogen in peripheral tissues (adipose, muscle, liver). Women with PCOS who do not ovulate have irregular, non-cyclic bleeding patterns. Bleeding occurs following chronic anovulation when endometrium growth is stimulated by estrogen in the absence of progesterone-mediated growth inhibition and progesterone-mediated development of stromal support. As a result, women with PCOS have variable endometrial bleeding episodes. Women with PCOS can present with menstrual dysfunction that presents as bleeding less than ≤ 8 times per year or ≥ 35 days between menstrual bleeding. In contrast, menses occurring at regular intervals, especially intervals between 24 to 35 days are predictive of regular ovulation (43-45).

The pathophysiology of PCOS is hypothesized to result from an increased pulsatile frequency of gonadotropin releasing hormone (GNRH) which selectively increases LH secretion (46). Women with PCOS often have mildly elevated levels of serum LH and an elevated LH to FSH ratio (47). LH stimulates theca cells in the ovary to produce androgens. FSH levels in women with PCOS are normal or suppressed and never reach the elevated threshold levels required in the early menstrual cycle to stimulate follicle growth (47, 48). Because of a relative insufficiency of FSH, androgens such as testosterone are ineffectively aromatized to estrogen by the GC. Androgens leave the ovary and are aromatized to estrogen in peripheral sites, such as fat, liver and muscle. Estrogen levels in anovulatory women with PCOS are typically constant at levels seen in the early to mid-follicular range in ovulatory cycles (48).

2.4.3 Polycystic Ovarian Morphology

In the scientific community, there is scant agreement on the morphological definition of a polycystic ovary (PCO). This controversy exists because the criterion put forth by the Rotterdam consensus, that the PCO contains over 12 follicles, has been characteristic of ovarian morphology of women who do not have PCOS. There are several studies that have challenged the Rotterdam criteria and have recommended revising the definition of PCO (2, 49).

Jonard et al. (50) investigated whether there was a rationale for increasing the threshold number of follicles to diagnose PCO from 10 to 15 follicles. It was found that increasing the threshold range of follicle numbers for a PCOS diagnosis had a higher specificity but not a higher sensitivity for using PCO as diagnostic criteria. The use of a threshold value of 12 follicles (the current standard) maintained the sensitivity at 75% and the specificity at 99%.

Dewailly et al. (49) suggested revisiting the threshold antral follicle count (AFC) in the diagnosis of PCOS as counts of 12 follicles per ovary resulted in an artificial inflation of the rate of PCOS in the population. It was suggested that advances in ultrasound imaging technology from the time of the Rotterdam consensus in 2003 would allow for improved visualization of follicles, especially of follicles in smaller diameter ranges (1 mm to 2 mm range). It was postulated that use of the Rotterdam definition of the PCO when there was a higher visual acuity for small follicles resulted in artificial inflation of diagnoses of women with PCOS (2, 49). Therefore, a higher threshold of the number of follicles to define PCO would represent a more accurate means to diagnose PCOS. In addition, real time ultrasonography was used to count the number of follicles when defining the PCOS criterion for the Rotterdam consensus. Dewailly noted that detailed post-hoc analyses using tracking software allowed for follicles to be counted that were previously uncounted. It was suggested that post-hoc analyses also led to an artificial inflation in follicle number using the definition from the Rotterdam consensus (2, 49) . Dewailly therefore assessed follicle numbers in a clinical population of infertile women using a cluster analysis. The cluster analysis consisted of counting follicle numbers in the lowest "cluster" of the non-PCO group and highest follicle numbers in the PCOS group. Dewailly used the cluster analysis to recommend a threshold of greater than 19 follicles for the diagnosis of the PCOS (49).

In 2013, Lujan et al. (2) recommended that the threshold follicle number of a PCO be increased to 26 follicles. In contrast to the work completed by Dewailly, Lujan recruited women of normal reproductive age (18 years to 35 years) with and without PCOS that did not present with infertility. Real-time imaging and post-hoc analyses were used to characterize follicle number. Women without PCOS who did not have infertility typically had greater follicle numbers than the control group with infertility that were evaluated by Dewailly. Inter-observer variability was evaluated by Lujan and used detailed post-hoc analysis to derive the antral follicle count. The sensitivity and specificity were 85% and 94% (49).

The use of anti-mullerian hormone (AMH) has been suggested to be a more reliable and less subjective marker of PCO morphology than use of the AFC. Anti-mullerian hormone is made by GC present in growing follicles until they reach about 8 mm in size. The production of AMH declines when a follicle is selected for dominance. Measurement of AMH compared is considered to be an increasingly reliable measure of ovarian follicle reserve. AMH levels are less prone to fluctuations and may also be less subject to inter-observer variability compared with the assessment of AFC and therefore be easier to reproduce during multi-center trials (51).

2.4.4. Antral Follicle Dysfunction in PCOS

Polycystic ovaries are characterized by a (2 to 3 fold) increase in antral follicle number compared to non-polycystic ovaries. Polycystic ovaries are also characterized by theca cell hyperplasia and concomitant thickening within the ovarian cortex (52, 53). When observing the PCO morphology in women with PCOS, it is important to distinguish between women who are ovulatory and anovulatory even though there is a greater tendency toward shared morphological characteristics. Gross morphology of anovulatory PCO consists of multiple 2 to 8 mm diameter antral follicles. The follicle sizes are consistent with follicular arrest prior to the pre-ovulatory stage (54). Granulosa cells found in the follicles of women with PCOS are steroidogenically competent. The GC from anovulatory women with PCOS show *in vitro* evidence of increased estradiol and progesterone production when compared with GC from follicles of similar size from ovulatory PCO and non-PCO women (55-57). In contrast, steroidogenesis by theca cells is abnormal in both anovulatory and ovulatory PCO suggesting an intrinsic abnormality in theca cell function (54). The overactive theca cells secrete increased amounts of androstenedione, 17-α-hydroxyprogesterone and progesterone compared with normal ovaries. Antral follicles in ovulatory PCO hypersecrete androgens but do not secrete excessive estrogen whereas antral follicles from anovulatory PCO have an increased secretion of both androgens and estrogen (58).

Androgens appear to influence the number of follicles in the ovary. In primates, androgens stimulate early follicle growth (54, 59). Androgen treatment in monkeys increases the number of primary, preantral and small antral follicles, and the proliferation of GC. Female rhesus monkeys exposed prenatally to excessive levels of testosterone or dihydrotestosterone (DHT) exhibited ovulatory dysfunction in adult stages, presenting with enlarged, hyperandrogenic polycystic ovaries (54).

Hyperandrogenism is unlikely the major cause of anovulation in PCOS; however, increased androgen levels may contribute to anovulation by inducing an abnormal environment that affects follicle maturation (54). The mechanism for androgens to induce abnormal follicle maturation in PCOS is taken from evidence of folliculogenesis and ovulation in non-PCOS women. In a typical ovulatory menstrual cycle, GC in the dominant follicle acquires LH receptors and the ability to respond to LH only in the mid-follicular phase. LH stimulates steroidogenesis and triggers terminal differentiation and arrest of follicle growth in a pre-ovulatory follicle. The arrest of follicle growth is thought to occur by exceeding a threshold level of intracellular cyclic AMP (cAMP) which is triggered by the LH surge. Possible mechanisms of follicular arrest include the hypersecretion of LH, insulin, and androgens and the production of supra-physiological intracellular concentrations of cyclic cAMP in the GC. In women with PCOS, the premature arrest of follicular growth is unlikely to be due solely to excessive LH secretion as many women have normal serum concentrations of LH. However, a synergistic effect of insulin and LH to stimulate estradiol and progesterone secretion could augment LH-induced steroidogenesis by GC (60). The synergistic effect of insulin on LH also may be due to premature acquisition of LH receptors prior to the mid-follicular phase. In non-PCO or in ovulatory PCO, the GC secretes estradiol in response to LH when the DF has reached 9 to10 mm. However, in anovulatory PCO, LH stimulated secretion of estradiol and progesterone could occur from follicles that were as small as 4 mm. Antral follicles that were 6 mm to 8 mm in diameter produced levels of estradiol and progesterone *in vitro* that were similar to those observed in normal, preovulatory follicles. In addition, androgens may contribute to follicular arrest by augmenting cAMP production within GC. Androgens have a negative effect on follicle growth and maturation in PCOS (57).

2.4.5. Reasons for the Increased Follicle Population in the Polycystic Ovary

There are two reasons hypothesized for the increased follicle population found in PCO: increased follicle recruitment and decreased follicle atresia. In both anovulatory and ovulatory PCO, there appears to be an increased recruitment from the resting follicle pool in comparison with observations of ovaries in women without PCO. An increase in the follicle resting pool is determined by observing an increased number of both primordial and primary follicles in PCO (61). Hughesdon et al. (53) found approximately twice the number of primary and secondary follicles in PCO compared with non-PCO. The density of small preantral follicles in the ovarian cortex was significantly higher in women with PCOS. It was hypothesized that the increased follicle density was primarily comprised of primary stage follicles. This hypothesis was tested using morphometric analysis of human ovarian cortical biopsies taken from groups of women with and without PCOS. More than 90% of the follicles in both PCO and non-PCO ovaries were at the primordial and primary stage. Occasional secondary follicles were observed in both groups. One early antral follicle was observed in a non-polycystic ovary. The population density of small preantral follicles differed between the PCO and non-PCO groups. The lowest density of small preantral follicles was seen in the non-polycystic ovaries. An intermediate density was noted in the ovulatory PCO and anovulatory PCO had the most dense follicle population. There was also a significant difference in density of primary follicles among all the groups. The median density of small preantral follicles was six fold greater in anovulatory PCO than in normal ovaries. Polycystic ovaries had greater density of the primary follicle population.

Webber et al. (61) found an increase in both primordial and primary follicles in women with PCOS as well as an increased density of follicles when compared with women without PCOS. Further analysis into the proportion of primordial (resting) and primary (growing) follicles indicated that there was increased initiation of growth of follicles from the resting pool regardless of ovulatory status in women with PCOS compared with the non-PCO population. Webber et al. hypothesized different possible mechanisms for the higher density of primary follicles in anovulatory PCO: an excess of primordial germ cells to the fetal ovary and more mitotic divisions of the oogonia in the fetal ovary. Other hypotheses included enhanced assembly of somatic cells around the naked oocyte during follicle formation around the twelfth week of gestation or a decreased rate of loss of germ cells and the surrounding somatic cells in the PCO compared to non-PCO. Anovulatory women with PCOS displayed abnormalities in the later stages of antral follicle growth, demonstrating an arrest of follicular growth, typically at a diameter of approximately 5 mm to 8 mm. The proportion of atretic follicles did not differ between PCO and non-PCO. Therefore, the difference in follicle density was not attributable to a reduced atresia rate. There was a trend toward a higher proportion of atretic follicles in anovulatory PCO (61).

Webber et al. also studied the health of the follicles by observing follicle morphologies. Histologic features of an atretic follicle included a degenerated oocyte nucleus, uneven or folded nuclear membrane, vacuoles in the oocyte, and pyknotic nuclei in the GC (61, 62). The proportion of healthy primordial follicles was significantly lower in PCO compared with a non-polycystic population. A greater proportion of early primary follicles was observed in the ovaries of anovulatory and ovulatory women with PCOS and a reciprocal decrease in primordial follicles was also observed (61).

2.5 Insulin Resistance and Hyperinsulinemia

2.5.1 Insulin Action *in vivo*

Insulin stimulates glucose uptake in tissues such as adipocytes, skeletal and cardiac muscle, and suppresses glucose production by the liver (63, 64). Insulin also decreases lipolysis and thereby decreases the level of circulating free fatty acids (FFA) in the body (65). Insulin resistance (IR) can often be confused with the inability of the body to produce insulin. More insulin is needed to achieve glucose homeostasis. Insulin resistance is coined to describe the decreased ability of a certain amount of insulin to efficiently mediate glucose uptake, glucose production, and/or lipolysis (66). Accordingly, insulin resistance is characterized by increased amounts of circulating insulin (66, 67).

Fasting glucose levels reflect hepatic glucose production in the body. Fasting insulin levels are a reflection of insulin sensitivity, secretion, and clearance (68, 69). The "gold standard" measure for insulin resistance is the euglycemic clamp technique. This test assesses insulin-mediated uptake of glucose. In lean healthy individuals, skeletal muscle accounts for 85% of insulin-mediated glucose disposal (IMGD) (70). As fat mass increases, skeletal muscle insulin sensitivity decreases (71). The clamp test is completed during steady state conditions, and the amount of glucose that is infused into an individual reflects the amount taken up by the peripheral tissues (67, 70). Dunaif et al. (72) demonstrated that IMGD was decreased significantly (35% to 40%) in women with PCOS compared to controls matched for age and body composition. The difference in IMGD was similar to the pattern described in Type 2 diabetes mellitus (T2DM). Insulin-mediated glucose disposal (uptake into muscle) was also decreased in lean women with PCOS with normal glucose tolerance. Although the clamp test adequately assesses IR, its use is limited because it is expensive and there is a requirement for highly trained personnel and specialized equipment (67, 70).

Another reproducible, quantitative measure of whole body insulin sensitivity is the frequently sampled IV glucose tolerance test (FSIGT) with minimal model analysis. This technique uses a mathematical model to analyze glucose and insulin concentration time courses after a glucose intravenous injection (70). The FSIGT test allows a direct stimulation of pancreatic β-cells without having gastrointestinal factors as confounders. The FSIGT provides a dynamic image of glucose and insulin concentration and the given dose of glucose is known as well as its appearance in systemic circulation (70, 73). Other measures that correlate with the gold standard tests have been developed to approximate degrees of IR because the clamp and FSIGT are expensive and complex procedures. All tests to calculate IR are based on fasting glucose and insulin levels and include homeostatic model assessment (HOMA), fasting glucose:insulin ratio, and quantitative insulin index (74-76).

Homeostasis model assessment of insulin sensitivity is a simple alternative to measure IR. The HOMA score is calculated from the following formula: [fasting plasma glucose (mmol/L)*fasting serum insulin (pmol/L)/22.5] (77). Bonora et al. (77) found a strong correlation between HOMA score and glucose clamp technique results and supported the use of HOMA as an index for *in vivo* insulin sensitivity. A HOMA score of 2.6 or higher indicates insulin resistance (78).

Oral glucose tolerance tests (OGTT) are used to measure glucose and insulin sensitivity but have shown insensitivity to large changes in insulin (71). Oral glucose tolerance tests lack precision for a quantitative measure of IR compared with the clamp or FSIGT measures (76, 79). However, there is no validated, universally accepted clinical test to detect IR (80). The fasting glucose: insulin ratio can be useful as a screening test but a 75g OGTT is more sensitive than the glucose:insulin ratio in determining impaired glucose tolerance (75, 80).

2.5.2 How Insulin Resistance and Hyperinsulinemia Affect Women with PCOS

Basic physiological differences in women with PCOS may account for much of the increased IR and subsequent hyperinsulinemia. Hyperinsulinemia can affect the ovaries through ovarian and non-ovarian linked pathways resulting in disrupted ovarian hormonal balance and regulation. Disruption of the menstrual cycle and infertility are present in addition to IR stimulating thecal cell mediated androgen production (81, 82). Ovarian mechanisms include insulin stimulation of thecal cells. Non-ovarian linked pathways include LH enhanced pituitary pulse amplitude and suppression of SHBG formation in the liver. Suppression of SHBG would result in decreased binding of testosterone subsequently raising the FAI in conjunction with insulin stimulation of cytochrome P450c17α activity resulting in an increase in adrenal androgens. Another way IR manifests itself in women with PCOS is by the impaired glucose uptake into skeletal muscle. Impairment occurs at the level of the insulin signaling pathway with the down-regulation of phosphorylation of some key signal molecules. Down-regulation of these molecules results in an impaired expression of glucose transporter 4 (GLUT4) and decreased glucose uptake into cells (83). Insulin signaling in women with PCOS has also been characterized in fibroblasts. The cultured fibroblasts from women with PCOS have shown decreased insulin receptor binding or auto-phosphorylation which could reflect mutations of genes regulating these pathways (84).

The distribution of adiposity in women with PCOS can also contribute to IR. Women with PCOS typically present with a higher amount of abdominal obesity compared with obese women without PCOS when matched for body mass index (BMI). Approximately 35% to 50% of women with PCOS have truncal obesity with an increased waist circumference (WC) (85). Adipocytes in visceral fat may contribute to IR because the visceral adipocytes are more metabolically active (83). The incidence of IR is up to 20% in lean women with PCOS and can be greater than 40% in the obese PCOS population (72, 80). The clinical features of PCOS, such as oligomenorrhea or amenorrhea, anovulation, and phenotypic presentations of hyperandrogenism, such as hirsutism and acne are exacerbated with concurrent obesity (80).

2.5.3 Cellular Basis of Insulin Resistance in Women with PCOS

Women with PCOS exhibit glucose intolerance, IR independent of obesity, and fasting hyperinsulinemia that are consistent with reduced insulin sensitivity and T2DM (86). Insulin insensitivity has been recorded despite normal insulin receptor occupancy which may suggest a deficiency in the insulin signaling pathway (87). Differences in the adipocyte insulin receptor pathway have been seen in women with PCOS compared to women without PCOS. The pathway involves the tyrosine kinase and skeletal muscle insulin receptor substrate-1 (IRS-1) associated phosphatidylinositol 3-kinase (PI3-K) (87, 88).

Ciaraldi et al.(86) conducted tests on adipoctyes and skeletal muscle cells in women with PCOS to investigate the expression and function of insulin signaling molecules in women with PCOS with both normal glucose tolerance (NGT) and impaired glucose tolerance (IGT). Impaired insulin sensitivity was present in both systemic circulation and in adipocytes but was not reflected in GLUT4 expression in women with PCOS. There were no detectable metabolic differences in glucose transport in adipocytes *ex vivo*. Differences between control and PCOS women were detected *in vivo*. In adipoctyes from the control population, 0.17 nm of insulin attained 50% of the maximal insulin effect. In adipocytes taken from women with PCOS, the same amount of insulin was able to stimulate only 15% to 20% of the final effect (86). Insulin action in skeletal muscle cells was studied by growing and differentiating them into myotubes which are phenotypically, morphologically, and metabolically similar to skeletal muscle cells donated by donors (89). Basal and insulin-stimulated glucose uptake into muscle cells was reduced by 50% for both NGT and IGT PCOS women compared to the controls. It was hypothesized that there was an intrinsic deficiency in the muscle cells of women with PCOS as the myotubes were not cultured in a hyperinsulinemic environment as found in women with PCOS. There was no noticeable difference between the NGT and the IGT PCOS myotubes *in* *vivo*. It was hypothesized that since the subjects were not postprandial (after having ingested a meal), the postprandial hyperglycemic state could not be evaluated to detect differences between the NGT and IGT groups. It was concluded that impaired sensitivity was apparent in the whole body context and was not intrinsic to muscle cells in women with PCOS. In addition, reduced insulin sensitivity in adipocytes could be an induced and reversible factor (86).

2.6 Obesity and Android Body Fat Distribution in Women with PCOS

Women with PCOS have increased android body fat distribution (BFD) characterized by mainly visceral and abdominal deposition of adipose tissue compared with age and BMI matched women (90). Women with PCOS are often hyperandrogenemic and hyperandrogenemia also results in visceral fat deposition. Studying the individual elements found in women with PCOS is difficult because it is difficult to isolate the effects of different confounders such as obesity and IR. Women with PCOS have a tendency to be overweight or obese (38% to 88%) and have a characteristic distribution of body fat around the abdomen (83). The development of obesity in women with PCOS is related to possible genetic predisposition to obesity and behavioural environmental factors such as poor diet and reduced exercise. Barber et al. (83) have put forward the suggestion that it is the development of obesity that unmasks the biochemical and clinical abnormalities of PCOS. Further evidence is taken from the finding that modest weight loss (5% to 10 %) results in a significant decrease in IR and hyperandrogenism in conjunction with an increase in menstrual cyclicity and ovulation. The nature of IR in women with PCOS is multi-faceted and multi-factorial. Studies evaluating the effect of insulin sensitizing medications have demonstrated there was a tendency to improve reproductive function and resolve the physical characteristics that result from hyperandrogenism (83).

2.6.1 Android Body Fat Distribution

An android BFD is seen more frequently in women with PCOS when compared with women without PCOS. Android BFD is characterized primarily by visceral and abdominal deposition of adipose tissue (83). Increased android BFD is associated with IR. The difference in IR in women with PCOS compared with controls is much less marked if women are matched for abdominal adiposity than matched for BMI (91). Exposure to high concentrations of androgens during fetal development is one possible mechanism of an android BFD (92). Studies with prenatally androgenized female rhesus monkeys show greater abdominal adiposity as compared with controls. Although hyperinsulinemia may play a role in the development of an android BFD in women with PCOS, it is difficult to determine a causal relationship. Android BFD contributes to hyperandrogenemia through increasing insulin resistance and the consequential hyperinsulinemia leads to the increase of android BFD. The android BFD causes and is a consequence of itself. This cycle can be disrupted by dietary/exercise interventions and insulin sensitizing medications (83).

2.6.2 Measuring Body Fat

It is a challenge to accurately measure body fat. Fat depots are mostly subcutaneous or intra-abdominal. These depots can also reside among and inside muscles (93). Recently, waist to hip ratio (WHR) and waist circumference (WC) both have been positively correlated with estimating abdominal fat. Waist circumference was determined to be more accurate than WHR (94, 95). It is better to consider both BMI and WC for a more accurate measure of visceral fat and cardiovascular risk although BMI has been widely used as the sole measure to evaluate body fat content (96, 97). The most accurate technique for measuring abdominal adipose tissue *in vivo* is computed tomography (CT). However, there is limited access to CT imaging equipment, increased exposure to ionizing radiation and the technique is expensive. Dual energy x-ray absorptiometry (DEXA) relies on the differential absorption of x-rays to distinguish different body tissues with a minimal exposure to radiation and is a comparable and validated alternative to CT scanning (98).

2.6.3 Clinical Observations Related to Abdominal Adiposity

Toscani et al. (93) investigated the influence of androgens on IR and central obesity in overweight or obese hirsute women with or without PCOS. They also tested the reliability of the sum of truncal skinfolds (subscapular, suprailiac, and abdominal) in estimating truncal adiposity. Hirsute patients with PCOS had a higher percentage of truncal adiposity, greater testosterone levels, and a higher FAI than women of similar age and hirsutism without PCOS. Women with PCOS also had increased WC, sum of trunk skinfolds, and DEXA- measured trunk fat. There was a strong correlation observed between the DEXA trunk fat measurements, WC, and trunk skinfold measurements. Trunk skinfold measurements were easier, cheaper, and readily available to perform; however, it was highly dependent on the individual, type of caliper, and the body composition of the person being measured. Waist circumference was suggested as a single, simple measurement that had a high correlation with DEXA measured trunk fat and had less variability than trunk skinfold measurements. Whole body DEXA was used to determine whether there was a greater amount of abdominal fat in women with PCOS than in weight-matched controls. The insulin levels and sensitivity in both groups were also assessed. Women with PCOS exhibited increased levels of testosterone and higher degrees of IR, independent of BMI. Body mass index was not found to be a predictor of central adiposity. Overweight and obese women with PCOS also had significantly higher WC, central abdominal fat, and serum insulin levels than women of a normal weight with PCOS. Higher insulin levels and reduced sensitivity were present in the entire PCOS population indicating that abdominal fat was not necessarily the only determinant of hyperinsulinemia in women with PCOS (93).

Carmina et al. (99) conducted a study to determine the relation of total and abdominal fat and IR in women with PCOS compared with weight-matched controls. Women with PCOS had higher testosterone and insulin levels, total fat, and trunk fat measured using whole body DEXA, despite similar values in BMI . Women with PCOS also had a significantly higher WC, percent trunk fat, abdominal (R1) fat quantity and percent R1 fat. Women with PCOS had similar quantities of trunk and total body fat compared to their weight-matched controls; however, they had an increased quantity of abdominal fat. Body mass index and WC measurements were not predictive of central abdominal fat excess. Overweight women with PCOS with increased abdominal fat also had significantly higher values of serum insulin and lower insulin sensitivity than those with normal central adiposity. Women with PCOS with normal abdominal fat were matched for insulin and insulin sensitivity values to overweight controls but not matched to overweight controls with increased abdominal fat. Hyperinsulinemia and IR found in women with PCOS seem to be a consequence of abdominal obesity even if abdominal obesity was not the only determinant. Women with PCOS with decreased abdominal fat content still showed signs of hyperinsulinemia and IR. It was difficult to determine whether IR was the cause or consequence of the android BFD (99).

Hutchinson et al. (100) conducted an investigation into the effect of a 12 week structured exercise program on IR and body composition in overweight and obese women with and without PCOS. Abdominal visceral fat was measured using CT scanning. After adjusting for age, abdominal adiposity was higher in women with PCOS. Abdominal adiposity also correlated independently with IR. The 12 week exercise program resulted in lower IR, abdominal adiposity (by 11%), and triglycerides despite weight maintenance in women with PCOS. Abdominal adiposity was an independent predictor of IR, dyslipidemia and cardiovascular risk factors. Hutchinson et al. suggested that weight loss should not necessarily be the sole focus of exercise in PCOS because exercise has been shown to increase the cardiometabolic health profile without weight loss.

2.7 The Relationship between Hyperandrogenism, Hyperinsulinemia, and Increased Android Body Fat Distribution in Women with PCOS

2.7.1 Androgens and Insulin Resistance

Hyperinsulinemia contributes to hyperandrogenemia in women with PCOS in several ways: 1) enhancing LH stimulated androgen production in theca cells; 2) decreasing the amount of SHBG, subsequently increasing the levels of free testosterone; and, 3) binding of insulin to the insulin-like growth factor (IGF-1) receptors on theca cells which also stimulates androgen production (101-103). In obese women, it has been demonstrated that there is an adipocyte site-specific increase of the expression of 17β-HSD which is the main reductive isoenzyme responsible for conversion of androstenedione to testosterone. The increase in the conversion to testosterone can be attributed to increasing androgenicity corresponding with increasing BMI (104).

Hyperinsulinemia causes hyperandrogenism but hyperandrogenism does not cause hyperinsulinemia. Decreasing serum insulin levels by insulin sensitizing drugs lowers serum androgen levels (105). However, decreasing serum androgen levels by bilateral oophorectomies or with GNRH agonists does not affect circulating insulin levels (106, 107). Prenatal exposure to high androgens may cause a visceral adiposity deposition leading to IR and subsequent hyperinsulinemia (108).

Insulin resistance becomes more pronounced with increasing age in women with PCOS and can be more pronounced compared with healthy women. The increase in IR could be attributable to an increase in abdominal obesity as measured by BMI, WC, and WHR (109). Pasquali et al. (110) showed no changes in plasma insulin levels and OGTT curves for women who did not experience change in BMI, WC, or WHR. There was a decrease in circulating androgens in women with PCOS as they aged and this change was more pronounced compared with normal women. The decline in androgen levels was accompanied by a decrease in the prevalence of the classic phenotypic presentation of PCOS (hyperandrogenism, anovulation, and PCO) and an increase in the phenotype that contained anovulation and PCO, but not hyperandrogenism (109).

2.7.2 Effects of Androgenization in Animal Models

We were unable to locate any published studies determining the effect of androgen signaling in women, however, the relationship has been studied in rats (111). Androgenizing female rats resulted in a decrease in whole body insulin sensitivity in intact and ovariectomized animals (112). The reduction in insulin sensitivity was not associated with increased free fatty acid or glycerol levels which led to the interpretation that increased lipolysis was not the cause of decreased insulin sensitivity (113). It has been postulated that androgenization deregulates post insulin receptor signaling events although the specific mechanisms have not yet been elucidated (114).

2.7.3 Adipocyte and Steroid Metabolism

Adipocytes are also involved in steroid metabolism and inter-conversion of steroidal hormones. Sex hormones, such as testosterone and estrogen, are derived from cholesterol and are steroid hormones. The first and the rate-limiting step in the biosynthesis of all steroid hormones is the conversion of cholesterol to pregnenolone. All sex steroids are produced through the

DHEA conversion to androstenedione and subsequently to testosterone and estradiol by 17β-HSD and aromatase, respectively. Approximately half of the testosterone in female circulation is secreted directly from the ovaries and the adrenal glands. The remainder of the conversion of androgens occurs peripherally. Testosterone is further metabolized in target tissues, such as adipocytes, into the more potent form, called 5α-dihydrotestosterone by the enzyme 5α-reductase or into estradiol by aromatase. The increase in abdominal adiposity in women with PCOS results in increased peripheral conversion of androgens into DHT or estradiol. Peripheral conversion of androgens leads to unopposed estrogen levels causing endometrial growth and proliferation, possibly leading to endometrial hyperplasia and cancer (83).

2.7.4 Adiposity and Insulin Resistance

Weight gain in women with PCOS is associated with increased insulin resistance. Increased abdominal fat accumulation is correlated with reproductive dysfunction (115). It follows that a loss of abdominal fat is correlated with resumption of ovulation (116).Weight loss is linked to increased insulin sensitivity, resumption of menstrual cyclicity, increased ovulatory function, and increasingly healthier metabolic profiles. It is difficult to isolate the sole effects of hyperinsulinemia as increased adiposity is a confounding variable in women with PCOS (83).

2.8 The Effect of Lifestyle Interventions in Women with PCOS

Lifestyle modification has been determined to be beneficial in women with PCOS. A modest amount of weight loss (5% to14%) improves cardiovascular risk factors, reduces abdominal fat, blood glucose, blood lipids, IR, serum androgens and increases menstrual cyclicity, ovulation, and fertility (81, 117). Reduction in insulin levels is considered to be the most beneficial determinant of reproductive health. Metabolic parameters are correlated with reduction in abdominal fat (85, 118).

2.8.1 Why Exercise Training?

Exercise training improves health parameters such as decreasing abdominal fat and increasing insulin sensitivity. Exercise prevents development of cardiovascular disease (CVD), Type 2 diabetes mellitus (T2DM), decreases morbidity, and mortality in addition to having psychological benefits (81). Long-term exercise is beneficial for long term weight management and maintenance. The combination of exercise and a healthy diet is most effective for losing and maintaining weight (119). Aerobic exercise improves CVD risk markers and body composition independent of weight loss in overweight or obese individuals (120). Resistance training on its own is beneficial for improving sensitivity to insulin as well increasing the amount of lean body mass, thereby increasing the basal metabolic rate (121). The combination of aerobic and exercise training reportedly has a synergistic effect on improving metabolic parameters, IR, reproductive functionality, and reducing abdominal fat in people that are obese (122, 123).

2.8.2 Exercise Training in PCOS

It is important to establish whether women with PCOS have physical limitations different than clinically healthy women. There were no differences reported in three studies assessing maximal aerobic capacity (VO_2max) in women with PCOS compared to age and BMI matched controls (124-126). VO_2max usually has an inverse relationship with IR (81). Orio et al. (127) found that there was a decreased maximal and submaximal cardiorespiratory response in young overweight women with PCOS. In the study conducted by Orio et al., the fasting insulin concentrations were much higher than in the other studies assessing VO_2max in women with PCOS (20.2 IU/L vs. 15 IU/L). Increased IR could be the reason for the submaximal cardiorespiratory response in this population although the specific mechanism of action has not yet been elucidated (127).

Thomson et al. (116) conducted a 20 week study to assess the effect of diet only, aerobic training by itself, or aerobic-exercise training combined with a moderate hypocaloric weight-loss regimen on body composition, cardiometabolic and hormonal profiles, and reproductive functions in overweight or obese women with PCOS. Exercise combined with the diet did not provide any additional benefits compared with diet only in terms of weight loss. Exercise facilitated changes in body composition. A 45% reduction in fat mass and 60% better preservation of fat-free mass was observed. There was a significant difference in body fat percentage, fat mass, and free fat mass in the diet and exercise groups compared to the diet only group. There was no difference observed among the exercise group (aerobic or aerobic and resistance training). Waist circumference was reduced by 11.1% across all groups. There was an inverse relationship between SHBG and weight loss and a lower body fat percentage correlated with a decreased amount of testosterone. Almost half of the participants reported improvements in ovulation and/or menstrual cyclicity with no differences observed between treatments. The hormonal profiles of the women in the study, specifically insulin, showed no change over the intervention. The authors speculated that this was a reflection of the sensitivity of the assay. Most of the changes in health parameters were attributed to energy restriction and weight loss. Exercise improved the body composition and fat distribution of the participants which was more important in long-term weight loss management and sustainability.

Another study was designed to compare exercise and the role of physical function to IR in overweight and obese women with and without PCOS (124). Participants were asked to undergo a maximal exercise test, skeletal muscle function assessments, and blood tests. Aerobic exercise capacity was not related to PCOS, however, an inverse relationship was observed between VO_{2max} and IR. There was also an inverse relationship of VO_{2max} to WC. It was hypothesized that IR, not PCOS, increased the risks of CVD. The presence of PCOS did not limit an individual's ability to tolerate physical exercise. Women with PCOS with higher IR may have impaired cardiorespiratory function and muscle strength which could impact exercise tolerance (124).

Vigorito et al. (128) conducted a study to assess the effects of exercise training on the cardiopulmonary functional capacity in women with PCOS (120). Women were randomly allocated into a PCOS-T (trained) group and PCOS-UnT (untrained) group. The trained group underwent a 12 week structured exercise training program. There was a significant decrease in BMI, WHR, baseline insulin and WC in the PCOS-T group as opposed to the PCOS-UnT group. Structured exercise training programs were beneficial due to the increase in cardiopulmonary functional capacity, increased weekly energy expenditure score, BMI and WC reduction, improved IR and reduced chronic inflammatory state.

Palomba et al. (129) compared the efficacy of a structured exercise training (SET) program versus a hypocaloric diet on the reproductive function in obese PCOS patients with anovulatory infertility. Pregnancy rate was the primary end-point. The SET group undertook a 24 week SET program while the diet only group followed a 24 week hypocaloric hyperproteic diet intervention. The SET program and the diet intervention were equally effective in allowing for increasingly regular menstrual cyclicity and an increase in fertility. Conclusions about pregnancy rates could be not be drawn due to a lack of statistical power, but a tendency toward higher pregnancy rates was noted with the SET group. There were significant improvements in body weight, BMI, WHR, WC, FAI, serum testosterone and SHBG in both interventions. In the diet only group, the body weight and BMI were significantly decreased. The SET group exhibited a decrease in WC related to a decrease in visceral fat and an increase in muscle mass. Taken together, these observations indicated there were a variety of ways to improve IR that caused a hyperandrogenic profile in women with PCOS.

Hutchinson et al. (100) conducted an investigation into the effect of a 12 week structured exercise program on IR and body composition in overweight and obese women with and without PCOS. At screening, advice about lifestyle and diet interventions were given to all participants. The focus was on weight maintenance and not weight loss. Weight did not decrease significantly within groups. BMI was significantly reduced within the PCOS group. Waist circumference was reduced in the non-PCOS population. Total and abdominal fat mass decreased in both the PCOS and non-PCOS groups. In addition, IR and triglyceride components were reduced in women with PCOS but no changes were exhibited in the non-PCOS group.

It appears that exercise has a beneficial effect in women with PCOS in terms of reducing IR, improving fitness, body composition, menstrual cyclicity, and ovulation (100, 124, 128, 129). However, most intervention studies had small sample sizes, a high drop-out rate, and a low compliance rate, thereby decreasing their overall power. The challenges in doing a complete study could be a reflection of the psychological characteristics of the PCOS population. Behavioural characteristics may predispose women to factors that contribute to the PCOS phenotype (81). In addition, not all studies reviewed were randomized and/or they were looking only at short term effects of diet and exercise rather than long term sustainability. The design weaknesses reduced generalizability of the data sets (116).

2.8.3. Combined Dietary and Exercise Lifestyle Interventions in Women with PCOS

A systematic review was conducted by Moran et al. (130) on randomized controlled trials (RCT) that focused on the effect of lifestyle interventions (dietary, exercise, behavioural management, and/or combination of all three) compared to minimal treatment for women with PCOS. The outcomes measures observed included reproductive factors (pregnancy, menstrual cyclicity, ovulation, total testosterone, SHBG, clinical hyperandrogenism), anthropometric factors (weight, BMI, android BFD, WC, WHR), and metabolic factors (OGTT, fasting glucose and insulin levels, lipid profile). Out of 21 articles retrieved, 13 were excluded on the basis of a lack of a control group (n=9), not being a RCT (n=1), and no access to a full text article (n=2). Six studies were included in the systematic review (128, 131-135).

The number of participants in the studies that were included ranged from 11 to 90. The drop-out rate and its relation to the time period of the intervention were as follows: there was a 0% drop out at 12 weeks (128, 133), 18% at 24 weeks (135), 25% at 24 weeks, 35% at 48 weeks (133), and 43% to 46% at 16 weeks (131, 134). The interventions were physical activity interventions (128, 131, 134) or combined dietary and exercise interventions (132, 133, 135). There was no clear benefit on reproductive factors such as pregnancy, menstrual cyclicity, or ovulation. There was no evidence for decreased FAI indicating that effects of lifestyle treatments on hyperandrogenism should be carefully examined. Lifestyle interventions improved anthropometric measurements such as a reduced endpoint weight (7% weight change). No differences were observed in OGTT, fasting glucose, or lipid profiles of women with PCOS undergoing the lifestyle interventions compared with minimal treatment (136). It was difficult to extrapolate the effect of combined dietary and exercise interventions in women with PCOS due to the high drop-out rate, low compliance rate and low statistical power.

2.8.4 Metformin

Metformin is currently used as an oral anti-hyperglycemic medication and is approved by the American Food and Drug Administration (FDA) and Health Canada for the management of Type 2 diabetes mellitus. Metformin use has been associated with decreased IR through inhibition of hepatic glucose production, decreasing intestinal glucose uptake and increasing peripheral insulin sensitivity. The observed treatment effects included increased menstrual cyclicity, improved ovulation rates and decreased circulating androgen levels. Combination therapies in women with PCOS using metformin are being used to determine if there is a synergistic effect of metformin on lifestyle interventions to enhance weight loss (137).

Ladson et al. (137) studied the combination therapy of metformin and lifestyle interventions in women with PCOS. The participants were randomly allocated into a metformin or a placebo group. Both interventions contained a dietary and exercise intervention component over a period of 6 months. The primary outcome measure was ovulation rate as determined by urinary progestin levels. The two treatment groups retained very similar baseline characteristics such as similar drop-out rates, racial distributions, hormonal profiles, ultrasonographic measures and metabolic profiles. There were no significant differences in the outcomes (i.e., primarily ovulation rates, but also hormonal and metabolic profiles) between the metformin and placebo arms. However, the study had limited power due to the drop-out rates. Women in the metformin arm lost more weight. It was hypothesized that the insulin sensitizing nature of metformin would make it easier for women to respond positively to diet and exercise interventions although metformin did not necessarily contribute to the effect of diet and exercise in women with PCOS. This hypothesis would lend support to circumvent the high drop-out rate and the low motivation and sustainability rate in such kinds of interventions.

Hoeger et al. (133) carried out a pilot study to evaluate metformin therapy or lifestyle modification and a combination of the two therapies over a 48 week period on ovulation induction and reduction of serum androgens in women with PCOS. Participants were assigned to four groups: 1) metformin 850 mg (n=9); 2) lifestyle modification with placebo (n=11); 3) lifestyle modification with metformin (n=9); and, 4) placebo only (n=9). The lifestyle interventions included counseling by a registered dietician and an exercise physiologist and a diet that was 500 to 1000 calories lower than their regular consumption. Significant weight reduction occurred in all the groups. The most significant weight reduction (7% to 10%) occurred in the combined group. Significant reduction in androgens (testosterone and FAI) was also noted only in the combined group. There was no significant change in fasting serum glucose concentrations nor were there significant differences in ovulatory events between the treatment and placebo groups. Subgroup analysis showed that women who lost weight with or without metformin were estimated to be 9 times more likely to become ovulatory (OR = 8.97, 95% CI 1.24-64.68). However, the small sample size resulted in a wide confidence interval which in turn decreased statistical power and generalizability. As observed in previous studies, weight loss has an influence on resumption of ovulation. The addition of metformin is another predictor of a tendency toward resumption of ovulation. A combined metformin and lifestyle intervention group is 16 times more likely to resume ovulation (OR=16.19, 95% CI = 4.36 - 64.22) (125).

2.9 PCOS and Long-Term Risk

2.9.1 Economic Burden

PCOS has a high prevalence (approximately 1 in 15 women) and a strong association with many metabolic and fertility abnormalities. Financial burden of women with PCOS on the healthcare system is an important issue to address (6, 138). Azziz et al. (6) established the prevalence of PCOS among women in the United States, assessed the prevalence of various morbidities associated with PCOS and then calculated the economic impact of treatment during the reproductive years. The morbidities included menstrual dysfunction and anovulation, endometrial hyperplasia and carcinoma, infertility, T2DM and hirsutism. The economic burden was evaluated by including the cost of the initial diagnosis and evaluation and treating each of the morbidities. The cost of treating PCOS exceeded $4 billion in the United States alone, taking into account care provided only during the reproductive lifespan while ignoring other complications associated and not counting repeat doctor visits. Approximately 40% of the burden was due to development and care for T2DM. An estimated 12.8% of the $12.6 billion spent on diabetic management for people under 45 years old has been attributed to women with PCOS (139, 140). Therefore, widespread screening and amelioration of PCOS are options to be highly considered by health professionals, especially in countries where health insurance is provided as a national expectation.

2.9.2 Endometrial Hyperplasia and Cancer

Women with PCOS need increasingly regular menstrual cycles to increase fertility outcomes when desired and to decrease the risk of endometrial hyperplasia or cancer. Women with PCOS who have irregular cycles still contain peripherally aromatized estradiol (in adipose tissue) initiating unopposed endometrial growth and proliferation. The hyperinsulinemic environment also down-regulates SHBG and increases the amount of free estradiol and testosterone in the periphery. Endometrial hyperplasia occurs in 35% of women with PCOS who do not have regular cycles (artificial or natural). Endometrial cancer represents 8% of all cancers occurring in women and the highest risk populations are women who are obese, have hyperinsulienmia, T2DM and PCOS. Insulin stimulates cell proliferation, promotes tumor angiogenesis and stimulates aromatase activity. All of these factors play a role in augmenting cellular proliferation of the endometrium leading to atypic differentiation which then may progress to cancer (22).

2.9.3 Type 2 Diabetes Mellitus

Insulin resistance is complex and has a variety of genetic and environmental bases in women with PCOS. Insulin resistance results in hyperinsulinemia which in turn affects lipid metabolism, protein synthesis, and androgen production. A sub-group of women with PCOS who have IR also go on to develop insufficient pancreatic insulin output or β-cell failure. Theresulting hyperglycaemia results in T2DM (141). Approximately 15% of postmenopausal women with PCOS have T2DM (142). The diagnosis also increases the risk of CVD by a factor of 4 to 7. The mechanisms by which T2DM increases CVD include directly increasing sympathetic activity, inducing abnormalities in endothelial function, indirectly impairing fibrinolysis, altered lipolysis, and induction of hypertension (143-147).

2.9.4 Metabolic Syndrome

Metabolic syndrome (MBS) has been classified by the presence of three or more of the following: 1) WC greater than 88 cm in females; 2) blood pressure (BP) of at least 130/85 mm Hg; 3) serum levels of high density lipoprotein (HDL) below <1.03 mmol/L for men and <1.3 mmol/L for women; 4) triglyceride (TG) levels >1.7 mmol/L; and, 4) increased fasting serum glucose levels at least >5.6 mmol/L. Metabolic syndrome is associated with a higher risk for developing T2DM as well as cardiovascular disease (148). Factors defining MBS are common in women with PCOS. A prevalence of MBS in women with PCOS was found to be approximately 46% in a cross sectional study conducted by Korhonen et al. (149).

Apridonidze et al. (143) conducted a retrospective chart review comparing the prevalence of MBS in women with PCOS compared to an age and BMI matched population. They also tried to determine if there were any phenotypic or hormonal differences between PCOS women with MBS and those without MBS. The prevalence of MBS in women with PCOS was 43%. The prevalence was higher than the age-adjusted rate of 24% obtained by the Third National Health and Nutrition Examination Survey (NHANES III). Higher prevalence persisted even when matched for age and BMI, the latter suggesting that obesity by itself did not account for the differences of the prevalence in the PCOS population. Women with PCOS and MBS presented more frequently with acanthosis nigricans, a phenotypic characteristic of IR, in addition to more severe hyperandrogenism (increased serum free testosterone, lower serum SHBG). Further evaluation showed that 91% of the women with PCOS presented with at least one of the abnormalities of MBS and 69% had at least two of the abnormalities present. Low HDL concentrations occurred most frequently (68%), followed by elevated BMI (67%), high blood pressure (45%), high triglyceride levels (35%), and high fasting glucose levels (4%).

2.9.5 Pulses and Their Relation to Type 2 Diabetes Mellitus

A pulse crop refers to a group of more than sixty different grain legume crops grown around the world. A pulse refers to the seeds of pulse crop, such as chickpeas, beans, peas, and lentils. They are typically made up of 20% to 25% protein and 40% to 50% starch. Pulses are also rich in dietary fibre. Pulses are a good source of starch, fiber, vegetable protein, and anti-nutrients, such as phytates, phenols, lectins, and enzyme inhibitors, some of which are natural α-glucosidase inhibitors (150). Jenkins et al. (146) documented an exceptionally low glycemic response when pulses were fed to healthy volunteers and demonstrated that pulses contained a carbohydrate component that was more slowly absorbed as compared to other grains (5, 151). The slower absorption led to decreased postprandial glucose and insulin releases and effectively lowered the glycemic index (GI) index of the diet (150).

The prevalence of T2DM has passed 13% of the global population and continues to be on the rise (152). Anti-hyperglycemic agents such as metformin prevent the development of T2DM and reduce the risk of microvascular complications such as new or worsening nephropathy or retinopathy (153, 154). However, anti-hyperglycemic agents cannot reduce macrovascular morbidities, such as death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke (154). α-glucosidase inhibitor acarbose is an anti-hyperglycemic agent and can reduce macrovascular morbidities (150, 151). Acarbose slows down the rate of absorption of dietary carbohydrate so that it is converted into slowly absorbed or 'lente' carbohydrates. Low glycemic index (GI) diets with acarbose are beneficial for glycemic control and greatly aid in decreasing either the incidence or severity of T2DM in addition to reducing cardiovascular disease risk (145).

Abeysekara et. al (155) observed a benefit of pulses in reducing total cholesterol and low density lipoprotein (LDL) in older adults, another age group at increased risk for CVD. The reduction in cholesterol, especially LDL, remains consistent with younger age groups as well (155).Sievenpiper et al. (5) conducted a systematic review and meta-analysis of RCT observing the effect of pulses on glycemic control in people with or without T2DM. The analysis of 41 RCT supported a beneficial effect of consumption of dietary pulses in T2DM. Glycemic effects varied by pulse with the strongest evidence for benefit linked to chickpeas. Both acute and chronic consumption of pulses resulted in reduced postprandial blood glucose and insulin releases.

Our review of the literature led us to the notion that the incorporation of a lower glycemic diet coupled with an increase in exercise should demonstrate positive health benefits in anthropometric features, reduction in risk factors for metabolic syndrome, and reduction in the symptoms present in PCOS. Therefore, the combined effects of pulse consumption and aerobic exercise should result in positive changes in metabolic and fertility outcomes in women with PCOS.

CHAPTER 3

OBJECTIVES AND HYPOTHESES

3.1 General Objectives

A larger scale clinical trial was designed to evaluate the short and long term effects of a pulse-based diet and aerobic exercise on infertility measures and metabolic syndrome risk in women with PCOS. The work presented in the thesis represents a subset of data from the larger scale clinical trial. The full trial protocol is included in Appendix A.

3.1.1. Specific Objectives

The objective of the work presented in the present analysis and work comprising the thesis was to compare anthropometric features (weight, BMI, WC, body fat percent), antral follicle count (AFC), fasting insulin levels, menstrual cyclicity, and abdominal adiposity before and after a dietary intervention.

3.2 General Hypotheses

To put the work presented in the current thesis in context, we hypothesized that for the large scale clinical trial, a pulse-based dietary intervention there would lead to: 1) a reduction in insulin levels; 2) improvement of markers of metabolic syndrome (i.e., reduced fasting glucose levels, triglycerides, blood pressure, abdominal fat, and increased HDL); and, 3) a change in physiological measures of PCOS. The measures assessed included decreased number of ovarian follicles, decreased menstrual cycle length and demonstration of menstrual cyclicity, increased levels of estradiol and progesterone, and decreased androgens.

We also hypothesized that women participating in the study would continue to consume pulses 6 to 12 months after the intervention. There would be a decrease in insulin levels, markers of metabolic syndrome, and symptoms of PCOS at 6 and 12 months post intervention compared with the pre-study indices. A pulse-based diet would also indirectly increase the quality of life of women with PCOS.

3.2.1 Specific Hypotheses

For the work presented in this thesis, we hypothesized that following the pulse-based dietary intervention there would be a decrease in: 1) weight, BMI, waist circumference, and body fat percent; 2) antral follicle count; 3) fasting insulin levels; and, 4) abdominal adiposity. We also hypothesized that women participating in the study who had menstrual cycles that were unpredictable would notice that their menstrual cycles would be more predictable in timing after the intervention. The null hypothesis was that the intervention would have no effect on any of these factors.

CHAPTER 4

MATERIALS AND METHODS

4.1 Study Participants

The work presented herein represents a subset of the endpoints being analyzed in an ongoing study titled Lifestyle Intervention for Women with Polycystic Ovary Syndrome: The Role of a Pulse-Based Diet and Aerobic Exercise on Infertility Measures and Metabolic Syndrome Risk (Appendix A). The full study is being conducted in collaboration among members of the College of Pharmacy and Nutrition, College of Kinesiology, and Department of Obstetrics, Gynecology and Reproductive Sciences in the College of Medicine at the University of Saskatchewan. The clinical trial was approved by the University of Saskatchewan's research ethics board (Appendix B).

Recruitment was performed using advertisements placed in newspapers and online venues and with posters placed in clinics and hospitals across the city. The advertisements were seeking women of reproductive age (18 to 38 years) with concerns about irregular or absent menstrual cycles, difficulty losing weight, and excess hair growth.

All participants signed a consent form prior to starting the study (a copy can be found in Appendix A). A diagnosis of PCOS was made with the identification of at least two of the three diagnostic criteria by a clinician specializing in reproductive endocrinology . The diagnostic criteria were: 1) irregular or absent menstrual cycles as defined by a history of cycles >35 days in length; 2) hyperandrogenism as defined by a Ferriman and Gallwey score of >6 or hyperandrogenemia; and, 3) polycystic ovaries (PCO) as defined by >25 follicles visualised upon transvaginal ultrasound (TVU), with no follicle >10 mm or corpus luteum (2, 15).

A diagnosis of PCOS was excluded in women with the following conditions: 1) taking anti-seizure or anti-psychotic medications known to induce development of PCO; 2) untreated hyperprolactinemia or thyroid disease; or, 3) excessive adrenal androgen production confirmed by a diagnosis of congenital adrenal hyperplasia or an adrenal tumor. Women were excluded from the study if they were using reproductive hormones, including contraception or fertility medications for a period of at least 3 months before the start of the study. Other exclusion criteria included: 1) a medical condition limiting the ability to exercise to 60% of maximal heart rate; 2) an inability to consume a pulse-based diet (allergies or intolerances); 3) an uncontrolled medical condition that interfered with ovarian or system hormone production; 4) pregnant or breastfeeding state; and, 5) residency outside of the local geographic area.

One hundred and sixty seven women (n=167) expressed interest in participating in the study. Eighty five women (n=85) were either excluded following the exclusion criteria or decided they were no longer interested in participating prior to any diagnostic visits. Forty nine women (n=49) who completed all of the initial measures (diagnostic visit, diagnostic bloodwork, diagnostic ultrasound, OGTT, DEXA, and diet instruction) were randomized either into the group receiving the pulse-based diet or into a control group following the National Cholesterol Education Program therapeutic lifestyle changes diet (TLC). Both dietary groups participated in an aerobic training program. The inclusion of the TLC arm (as the control group) and the aerobic training program were considered the most ethical approach to our study design (i.e., the TLC diet and aerobic program were considered “standard of care” for women with a condition negatively affecting health). For this ethical reason we did not have an untreated control group.

4.2 Diet and Exercise Program

All participants were asked to attend a 90-minute dietary education session with a registered dietician to learn about the composition of the TLC diet which used meat-based protein sources. All participants followed the TLC diet during the first two week “lead-in period”. Following the “lead-in period”, participants were randomized to either a 16 week pulse-based diet or to continue the TLC diet for 16 weeks. Pre-intervention measures were obtained before the start of the 16 week diet and exercise intervention and post-intervention measures were obtained after week 12 of the intervention was completed, depending on the menstrual bleeding pattern of the individual.

Participants randomized to the pulse-based group received 2 pulse-based meals each day for 16 weeks. The pulse-based meals were prepared with dry peas, lentils, chickpeas, or beans. The meals contained approximately 90 g of dried green peas, 225 g chickpeas or fava beans, or 150 g lentils. Participants were asked to follow TLC guidelines for breakfasts and snacks.

All participants were asked to follow an exercise program for the 16 week duration of the intervention. The exercise program involved 5 sessions of aerobic exercise per week; 3 sessions of supervised exercise training and 2 sessions of unsupervised at-home exercise were to be undertaken. The supervised exercise training occurred in the College of Kinesiology physical activity complex. Exercise was completed on a treadmill, rowing machine, elliptical trainer, or exercise bike. Participants were asked to exercise for 45 minutes each day at an intensity of at least 60% of their age-predicted maximal heart rate (i.e., 220-age).

4.3 Measurements of Demographic, Androgenic, and Anthropometric Features

4.3.1 Age

The participant age was calculated at the time of the first diagnostic visit.

4.3.2 Height

Standing height was measured by placing the participant without footwear, arms hanging by the sides, feet together, against a meter stick attached to the wall. The participant was instructed to look straight ahead, stand as tall as possible, and take a deep breath while the measurement was taken. The measurement was taken by placing a set square on the head, depressing the hair to make firm contact with the top of the head. A mark was made at the level of the lower border of the square on the wall. The distance from the floor to the pencil mark was recorded to the nearest 0.5 cm (Figure 4.1a).

4.3.3 Weight

Weight was measured using an analog scale (Bridgeview, IL, Health-O-Meter; model 160KL) placed on a flat surface. The participant was measured without footwear and in light clothing. The weight was measured in kilograms to the nearest 0.1 kg (Figure 4.1b).

Figure 4.1. Measurement of standing height and weight (a,b). Placement of the participant against a wall and placing the set square on the head, while depressing the hair to make firm contact with the top of the head (a); and, measurement of weight by placing the participant on the analog scale (b).

4.3.4 Body Mass Index

Body mass index (BMI) was calculated from the recorded height and weight using the formula: [body weight (kg)/(height squared) (m^2)] (99).

4.3.5 Waist Circumference

Waist circumference was measured in accordance with the protocol provided by the Canadian Society for Exercise Physiology (CSEP). The participant's abdomen was cleared of all clothing and accessories. The participant was positioned with feet shoulder width apart and arms crossed over their chest in a relaxed manner. The WC was taken at the top of iliac crest as specified by the National Institute of Health (156). The top of the iliac crest was identified by palpating the right hipbone of the participant until the uppermost lateral border was located. A horizontal line was drawn at this landmark. An anthropometric tape measure was positioned directly around the abdomen with the inferior edge of the tape level with the landmarked point. A cross-handed technique was used to bring the zero line of the tape in line with the measuring aspect of the tape. The tape was positioned in a horizontal plane around the abdomen with enough tension to ensure that it was snug without causing indentation at the skin (Figure 4.2). The measurement was taken to the nearest 0.5 cm.

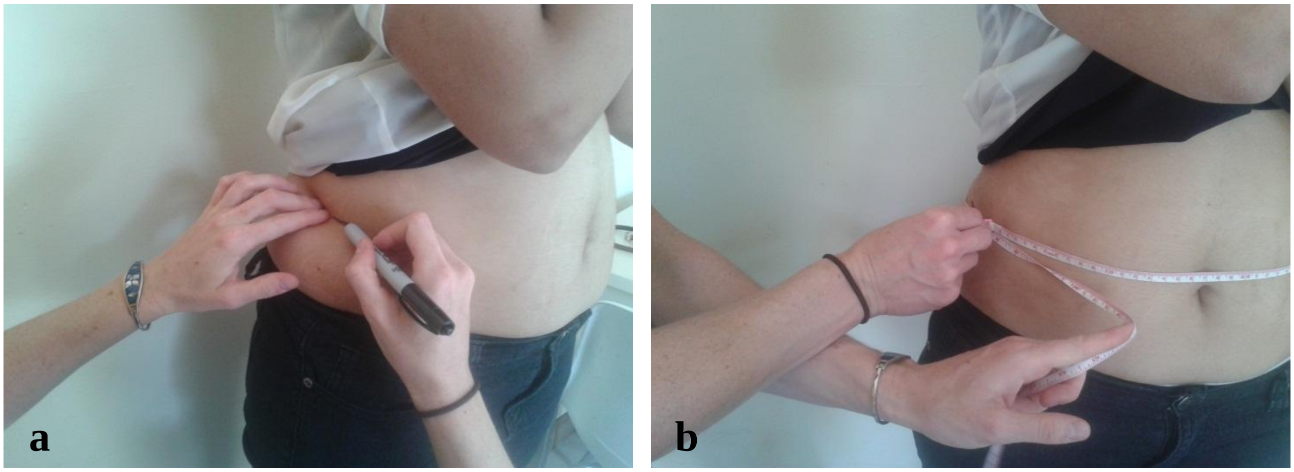

Figure 4.2. Measurement of waist circumference (a,b). Marking the top of the iliac crest after palpation of the top of the right hipbone (a); and, cross-handed technique used to bring the zero line of the tape in line with the measuring aspect of the tape using enough tension to ensure it is snug without causing indentation of the skin (b).

4.3.6 Free Androgen Index

Participants underwent clinical evaluation before the first diagnostic visit. The measures evaluated included serum levels of testosterone and SHBG and were analyzed by the Saskatoon Health Region. Blood was collected in a red top glass tube and was centrifuged after a complete clot was formed. Total testosterone was measured using the IMMULITE 2000 Systems Analyzers (Tarrytown, NY, USA) solid-phase, competitive chemiluminescent enzyme immunoassay. SHBG was measured using the IMMULITE 2000 Analyzers solid-phase two-site chemiluminescent immunometric assay. The free androgen index (FAI) was calculated by the following formula: [(total testosterone/SHBG)*100] (41).

4.3.7 Body Fat Percent

Percent body fat was calculated from measurements with the whole body dual x-ray absorptiometry (DEXA) scan before and after the intervention. DEXA technicians used a Hologic© Discovery W (Bedford, MA) machine for all scans (99). To prepare for the scan, the participant was cleared of anything made of metal and all footwear was removed. The participant was placed in a supine position on the DEXA scan table with the centerline marking dividing their body in half. The participant's head was placed approximately one inch below the horizontal line at the top of the table pad. The participant was positioned with their hands palms down and their arms extended beside the body, fingers together, with no limb overlap. The participant's feet were angled inwards with great toes touching; masking tape was used to hold the feet in position (Figure 4.3). The participant was instructed to not move while the whole body scan was in progress.

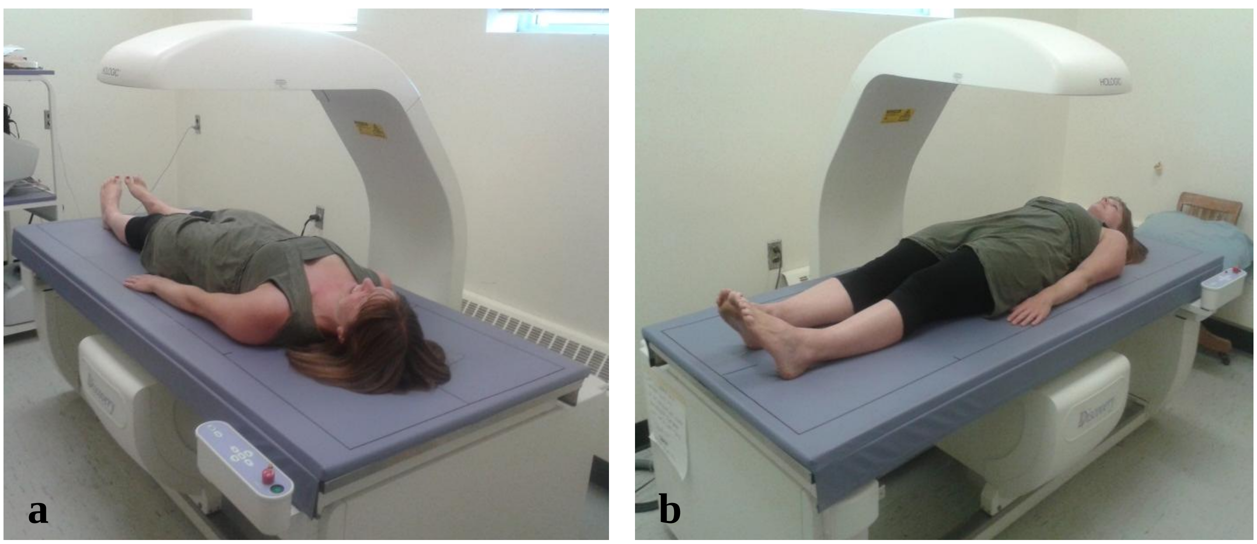

Figure 4.3. Photographs illustrating participant placement on the DEXA scan table (a,b). Placement of the head approximately one inch below the horizontal line at the top of the table pad (a); and, toes were located facing inward (b).

The images from the whole body scan was analyzed by the DEXA technician immediately using the Hologic© program. The images were sectioned as follows: left arm, right arm, neck, L1to L4, pelvic area, left leg, and right leg (Figure 4.4). The Hologic© program automatically calculated body fat percentage from the data collected from each (n=20) participant.

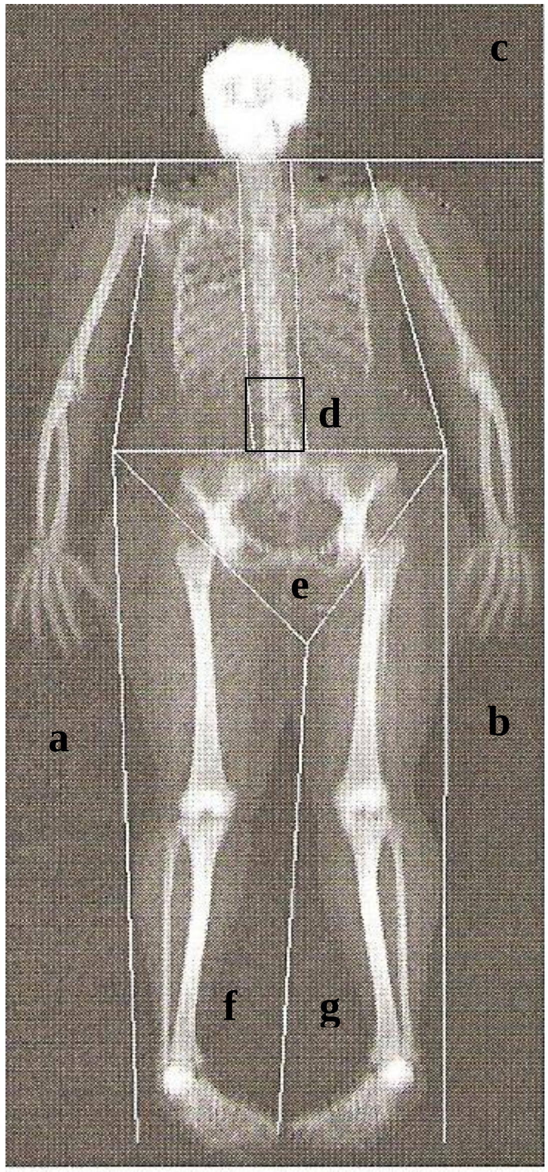

Figure 4.4. Whole body DEXA scan sectioned as follows: a) left arm; b) right arm; c) neck/head area; d) L1to L4; e) pelvic area; f) left leg; and, g) right leg.

4.4 Pelvic Ultrasound Examination

4.4.1 Data Collection

Transvaginal ultrasonography was used to examine each participant before and after the intervention (Ultrasonix RP, 6-9MHz curvilinear array transducer, Burnaby, BC, Canada and GE Voluson® 730 Pro, Voluson® Endocavity Transducer RIC5-9-D, Zipf, Austria). The reproductive organs of all participants were evaluated by two experienced ultrasonographers. The ultrasound examinations were conducted on cycle days 1 to 5 for women who had regular menstrual cycles. Women with irregular cycles had an ultrasound examination on a random day provided all follicles were below 10 mm in diameter (Figure 4.5). For our clinical trial, rigorous ultrasonographic criteria were defined as: 1) non-PCO ≤20 follicle; 2) borderline PCO ≥20≤30 follicles; and, 3) definite PCO ≥30 follicles. The PCO criteria reflected the high resolution ultrasound machines and detailed post-hoc analyses (2). Ovaries were examined in transverse and sagittal planes and images were captured as real-time cine-loop recordings and 3-dimensional images (GE Voluson®).

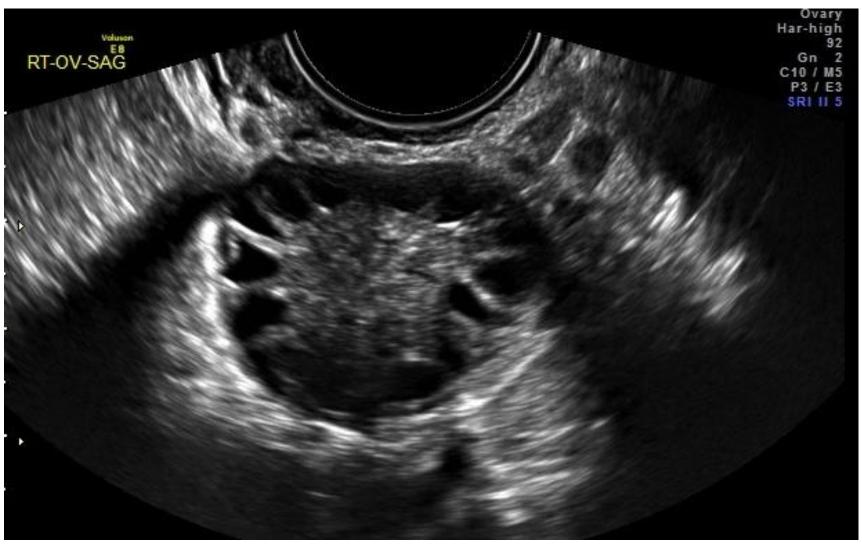

Figure 4.5. Ultrasound image of a polycystic ovary with characteristic "string of pearls" distribution of follicles around the edges of the ovary.

4.4.2 Antral Follicle Counts

Digital cine-loop recordings of each ovary were converted into DICOM file format and analyzed using Santesoft DICOM editor software (©Emannouil Kanellopoulus, Athens, Greece). The cine-loop in the transverse or sagittal plane with the best visual acuity for both the left and the right ovary per participant was chosen for analysis. The cine-loops were analyzed on a frame-by-frame basis in a single plane. Follicle diameters were measured in the widest plane in order of appearance as each frame was examined. (Figure 4.6). Care was taken to ensure that individual follicle positions were assessed so that there was no overlap from frame to frame when assessing the number follicle in each ovary. Each follicle was measured using the measuring tool found in the Santesoft DICOM editor. Measurements were taken in pixel units.

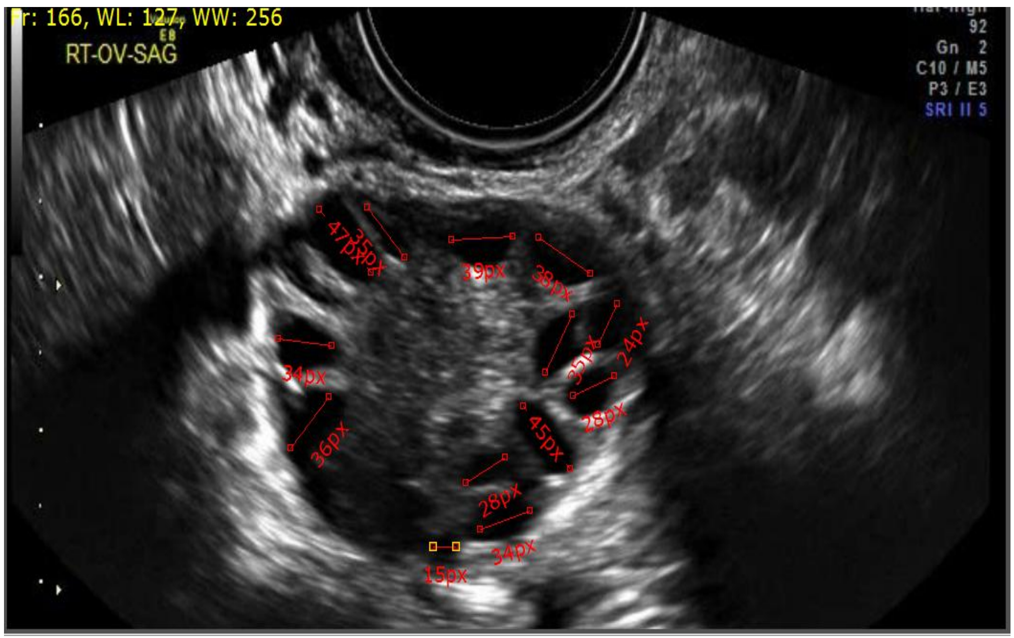

Figure 4.6. Ultrasound image of a polycystic ovary demonstrating the use of the measuring tool found in Santesoft DICOM editor to measure follicles in their widest planes in pixels.

Follicle positions were illustrated manually on a schematic illustration of an ovary in order of appearance (Figure 4.7). Follicle measurements (in pixels) were transferred digitally into an Excel spreadsheet (Microsoft Excel, Redmond, Washington).

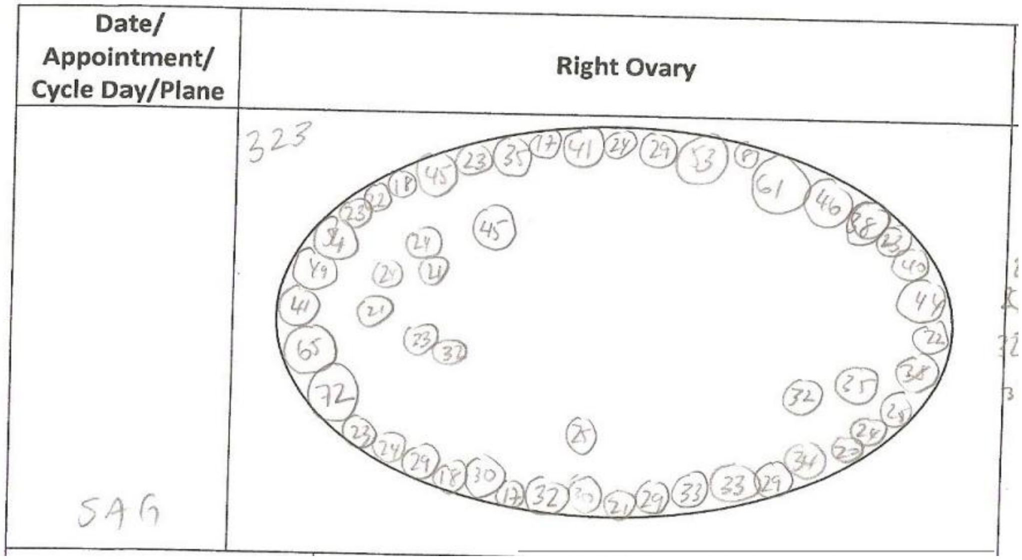

Figure 4.7. Schematic illustration of the follicle measurements in pixel units as drawn from the Santesoft DICOM editor.

To convert the follicle size from pixel units into millimeters (mm), the scale bar found on the left of the Santesoft DICOM editor was measured in pixels (Figure 4.8). The scale bar is an internal ultrasound reference scale and each notch was equal to 5 mm. The follicle size in pixels was converted to mm using the following formula: [follicle width in pixels*(mm length of scale bar/pixel length of scale bar)].

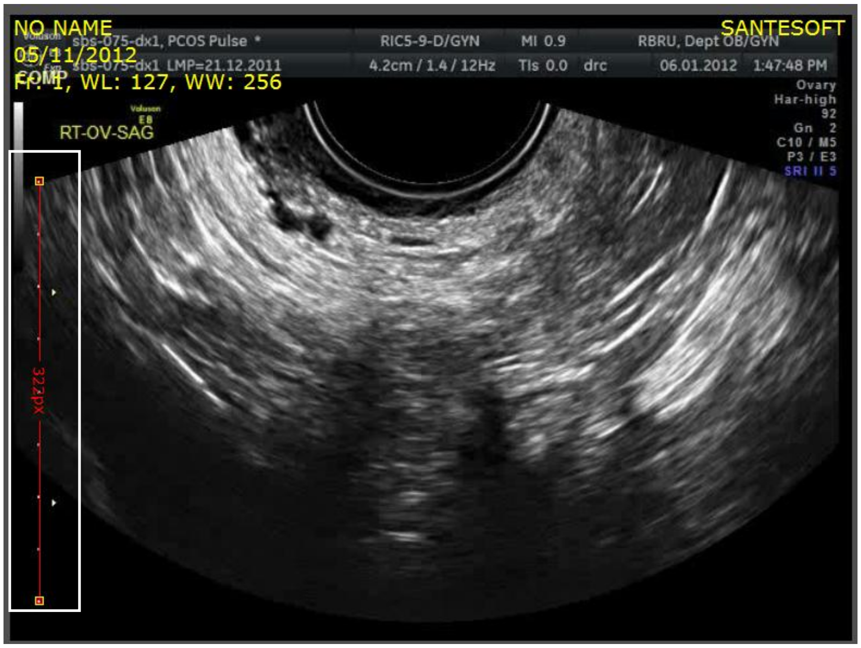

Figure 4.8. Measurement of the scale bar (left of image surrounded by white box) of the Santesoft DICOM editor in pixel units.

The number of follicles drawn was transferred to a spreadsheet and counted to determine a value for the antral follicle count (AFC) for the left ovaries (LO) and right ovaries (RO) for each participant before and after the intervention (RO n=20, LO =17) as per the standard operating procedure (SOP) in our laboratories (2).

4.5 Fasting Glucose and Insulin Levels

Fasting insulin levels were obtained at the beginning and end of the intervention during the fasting oral glucose tolerance tests (OGTT). The subjects were asked to fast for 10 to12 hours prior to the OGTT. Fasting blood was collected in a red top glass and a lavender EDTA plastic tube by a phlebotomist. The blood was analyzed by the Saskatoon Health Region laboratory using the IMMULITE 2000 Systems Analyzers solid-phase enzyme-labelled chemiluminescent immunometric assay (n=20).

4.5.1 HOMA Score

The HOMA score was calculated using the following formula: [fasting plasma glucose (mmol/L)*fasting serum insulin (pmol/L)/22.5] (77).

4.6 Abdominal Adiposity

Abdominal adiposity estimates were derived from a region analysis of the whole body DEXA scan. The region of interest (R1) was determined by evaluating the central abdominal region between the lateral iliac crests and the lowest rib margins and spanning the area of the first and fourth lumbar vertebrae (Figure 4.9) (99). The Hologic© program then automatically calculated the amount and percent of abdominal adiposity in R1 using a propietary algorithm (n=13). The change in adiposity during the study was derived from adiposity measurements taken before and after the intervention.

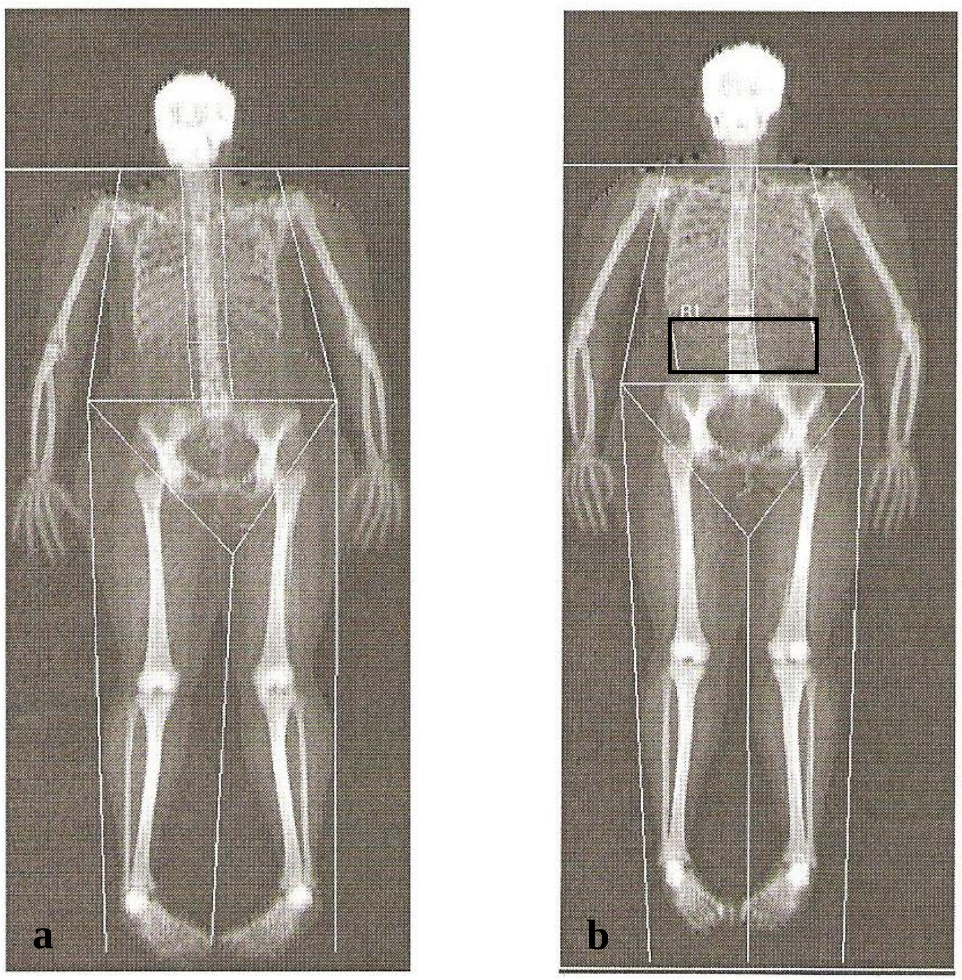

Figure 4.9. a) Whole body DEXA scan; and, b) R1determined by the area around the midpoint of the midline between the lateral iliac crests and the lowest rib margins and spanning the area of the first and fourth lumbar vertebrae.

4.7 Menstrual History

Menstrual histories were taken before and during the intervention. A menstrual cycle was defined as the start of one cycle (menses or menstrual bleed) to the start of another cycle. Participants were asked to recall their menstrual bleeding patterns 6 months before the intervention. The shortest period of time between menses and the longest period of time between menses was recorded. Menstrual bleeding was tracked during the intervention by informing the research associate about the date on which menses began. The average length between menses and the longest length between menses were calculated.

4.8 Statistical Analyses

The primary endpoints were the changes in participant weight, BMI, body fat percent, AFC, fasting insulin levels, menstrual cyclicity and abdominal adiposity after the intervention. Differences between the pulse and the TLC interventions were evaluated for each endpoint.

Non-parametric statistical analyses were used due to the small population. The Wilcoxon signed-rank tests (for dependent samples) were used to compare weight, BMI, waist circumference, body fat percent, AFC, fasting glucose and insulin levels, HOMA score, abdominal adiposity, and length between menses of pooled pulse and TLC groups before and after the intervention in order to evaluate an effect due to the intervention.

To evaluate the effect of a pulse-based diet, the mean difference between the pulse and TLC group was calculated by subtracting the measures after the intervention from the measures before the intervention. The mean difference, also known as the change variable, was analyzed using the NPAR1WAY procedure (for independent samples) to identify differences between the pulse and TLC groups. Alpha was set at 0.05. All statistical analyses were conducted using Statistical Analysis System (SAS, version 9.2, Cary, NC, 2012).

CHAPTER 5

RESULTS

Out of the 167 women who expressed interest in participating in the study, Eighty five women (n=85) were either excluded or decided they were no longer interested in participating prior to any diagnostic visits. Fourteen women were excluded based on living outside of the city, 23 women were no longer interested after reading the consent form, 20 women did not respond after initial contact, 7 women wanted to try to conceive immediately, 8 were excluded after they were not diagnosed with PCOS, and 6 women were on birth control. The other 21 women that were excluded were due to other various reasons. Forty nine women (n=49) completed all of the initial measures (diagnostic visit, diagnostic bloodwork, diagnostic ultrasound, OGTT, DEXA, and diet instruction) were randomized either into the group receiving the pulse-based diet or into a control group following the National Cholesterol Education Program therapeutic lifestyle changes diet (TLC). Twenty four women completed the 16 week dietary intervention to date (pulse n=13, TLC n=11). Sixteen women dropped out of the study at the intervention phase (pulse n=8, TLC n=8). The overall drop-out rate was 33%

There are different numbers of participants for each of the results due to several reasons: data was only available for 7 of the 11 TLC members as we did not obtain all of the data points of some of the participants in the beginning of the study. Abdominal adiposity could only be evaluated in 19 participants instead of 20 participants as the archived DEXA scans of one of the participants could not be located. The number of right ovaries (n=20) was different than the number of left ovaries (n=17) due to left ovaries being harder to visualize and subsequently, we were unable to do an antral follicle count on left ovaries in all participants. Abdominal adiposity could only be evaluated on 13 of the participants due to the inability to locate older archived DEXA scans.

A comparison of anthropometric features at baseline of all study participants in the pulse and the TLC group are presented in Table 1. There were no significant differences in anthropometric features between the pulse (n=13) and the TLC group participants (n=7) at baseline. Participants were similarly matched for age, weight, BMI, WC, and FAI. Body fat percentage tended to differ (p=0.09) between the groups.

Table 1. Demographic, anthropometric and androgenic measures at baseline of all participants by study group: pulse and TLC

	Pulse ± SD (n=13)	Range	TLC ± SD (n=7)	Range	p value
Age (years)	27.4 ± 5.65	19.0 - 36.0	28.3 ± 3.44	24.0 - 33.0	0.25
Weight (kg)	81.2 ± 15.08	56.3 - 102.8	90.4 ± 18.22	69.0 - 115.0	0.13
BMI (kg/m^2)	30.8 ± 6.45	20.7 - 41.6	33.7 ± 6.89	24.2 - 40.3	0.18
WC (cm)	102.0 ± 22.62	69.1 - 164.4	108.9 ± 16.24	87.8 - 125.0	0.12
Body Fat (%)	40.8 ± 7.22	25.0 - 51.0	44.4 ± 8.33	30.0 - 54.0	0.09
FAI	6.8 ± 5.37	1.6 - 16.9	5.4 ± 2.97	2.0 - 10.4	0.94

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The difference in the anthropometric features of pooled study participants before and after the intervention and the difference within the pulse and TLC group is presented in Table 2. Weight decreased from 83.0 ± 15.92 kg to 79.3 ± 14.12 kg ($p=0.002$). Body fat percent also decreased from 41.6 ± 7.51% to 39.1 ± 6.53% ($p=0.0004$). There were no significant differences detected in BMI or waist circumference. There was no significant difference of the pulse-based intervention detected on weight ($p=0.69$), BMI ($p=0.87$), WC ($p=0.56$), or body fat percentage ($p=0.51$) compared with the TLC group.

Table 2. The effect of the overall intervention and the pulse-based diet on anthropometric features evaluated using the Wilcoxon signed-rank test and NPAR1WAY procedure

	Mean Pre Intervention ± SD	Range	Mean Post Intervention ± SD	Range	Mean Difference Between Groups ± SD	p value of pooled groups	Mean Difference (Pulse)	Mean Difference (TLC)	SD of Mean Difference	p value of pulse vs. TLC group
Weight (kg; n=20)	83.0 ± 15.92	56.3 - 115	79.3 ± 14.12	55.5 - 106.0	3.8 ± 4.44	**0.002**	3.5	4.3	12.62	0.69
BMI (kg/m^2; n=20)	31.4 ± 6.48	20.7 - 41.6	31.2 ± 5.55	21.8 - 41.7	0.3 ± 3.62	0.81	0.4	0.1	12.62	0.87
WC (cm; n=20)	102.6 ± 21.32	69.1 - 164.4	97.3 ± 13.65	71.4 - 123.6	5.2 ± 20.25	0.23	7.3	1.5	12.62	0.56
Body Fat (%; n=19)	41.6 ± 7.51	25.0 - 54.0	39.1 ± 6.53	24.2 - 49.3	2.1 ± 2.21	**0.0004**	1.9	2.6	11.43	0.51

The effect of the overall intervention and the pulse-based diet on study participants on AFC in the right ovary and the left ovary, fasting insulin levels and abdominal adiposity are presented in Table 3. Antral follicle counts were decreased in the right ovary (p=0.04). Follicle number in the right ovary decreased from a count of 43 ± 14.9 follicles to 34 ± 12.9 follicles; however, a decrease in AFC was not observed in the left ovary (p=0.11). The difference between an ovary of the same participant before and after the intervention as seen on an ultrasound examination is presented in Figure 5.1. Fasting glucose levels did not show a significant difference. Fasting insulin levels decreased (p=0.02) in the pooled groups from 89.7 ± 69.05 pmol/L to 67.9 ± 54.02 pmol/L. The HOMA score also showed a decrease (p=0.02) in the pooled groups from 22.7 to 14.9. No change in abdominal adiposity was detected (p=0.88). There was no difference detected in AFC in either the right ovary (p=0.46) or the left ovary (p=0.40) due to the pulse-based diet. However, there was a tendency toward a change between groups in fasting insulin levels (p=0.07) and HOMA score (p=0.08). There was no difference in the abdominal adiposity between the groups (p=0.56).

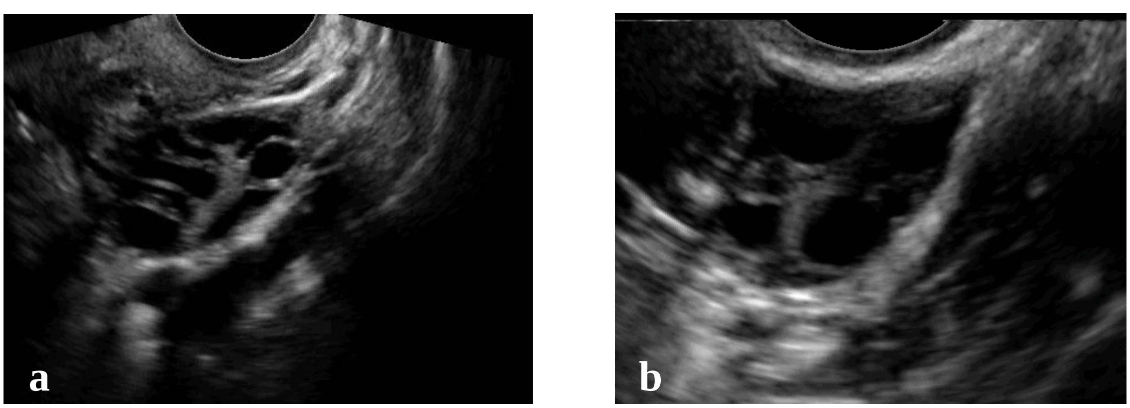

Figure 5.1. Ultrasound images of a left ovary in the transverse plane (a,b). Ultrasound image of a left ovary in the transverse plane before the intervention (a). Ultrasound image of the same left ovary in the transverse plane after the intervention (b).

Table 3. The effect of the overall intervention and the pulse-based diet on antral follicle count, fasting glucose and insulin levels, HOMA score, and abdominal adiposity before and after the intervention using the Wilcoxon signed-rank test and NPAR1WAY procedure

	Mean Pre Intervention ± SD	Range	Mean Post Intervention ± SD	Range	Mean Difference ± SD	p value of pooled groups	Mean Difference (Pulse)	Mean Difference (TLC)	SD of Mean Difference	p value of pulse vs. TLC
Antral Follicle Count (Right Ovary) (n=20)	43 ± 14.9	13 - 76	34 ± 12.9	13 - 66	9 ± 15.6	**0.04**	7	12	12.6	0.45
Antral Follicle Count (Left Ovary) (n=17)	38 ± 14.0	16 - 77	32 ± 14.1	7 - 68	6.5 ± 17.9	0.11	2	14	9.9	0.21
Fasting Blood Glucose Levels (mmol/L, n=20)	5.2 ± 0.860	4.0 - 8.4	4.87 ± 0.457	3.8 - 5.5	0.4 ± 0.87	0.09	0.5	0.3	12.6	0.94
Fasting Insulin Levels (pmol/L; n=20)	89.7 ± 69.05	14.4 - 268	67.9 ± 54.02	14.1 - 224	21.8 ± 53.25	**0.02**	37.0	-6.5	12.62	0.07
HOMA score (n=20)	22.7 ± 22.64	3.3 - 100.1	14.9 ± 12.17	2.4 - 49.78	7.79 ± 18.72	**0.02**	12.1	-0.2	12.62	0.08
Abdominal Adiposity (%; n=13)	34.7 ± 9.68	22.2 - 53.1	33.9 ± 8.25	21.5 - 49.5	0.85 \± 5.941	0.88	-0.42	1.65	6.831	0.56

The mean length of time and the longest time of time between menses is presented in Table 4. The average length of time of menses decreased from 71 ± 82.4 days to 39 ± 15.9 days after the intervention (p=0.04). The pattern of change in average lengths of time between menses is illustrated in Figure 5.2. The longest length of time between menses decreased from 91 ± 87.6 days to 43 ± 19.1 days (p=0.01) and is illustrated in Figure 5.3.

Table 4. The effect of the lifestyle intervention and the pulse-based diet on average length of time and longest length of time between menstrual cycles using the Wilcoxon signed-rank test and NPAR1WAY procedure

	Mean Pre Intervention ± SD	Range	Mean Post Intervention ± SD	Range	p value of pooled groups	Mean Difference (Pulse)	Mean Difference (TLC)	SD of Mean Difference	p value of pulse vs. TLC group
Mean Length Between Menses (days, n=16)	71 ± 82.4	29-365	34 ± 12.9	29-365	**0.04**	-11	-109	8.2	0.32
Longest Length Between Menses (days; n=16)	91 ± 87.6	27-85	32 ± 14.1	28-90	**0.01**	-28	-20	6.9	0.48

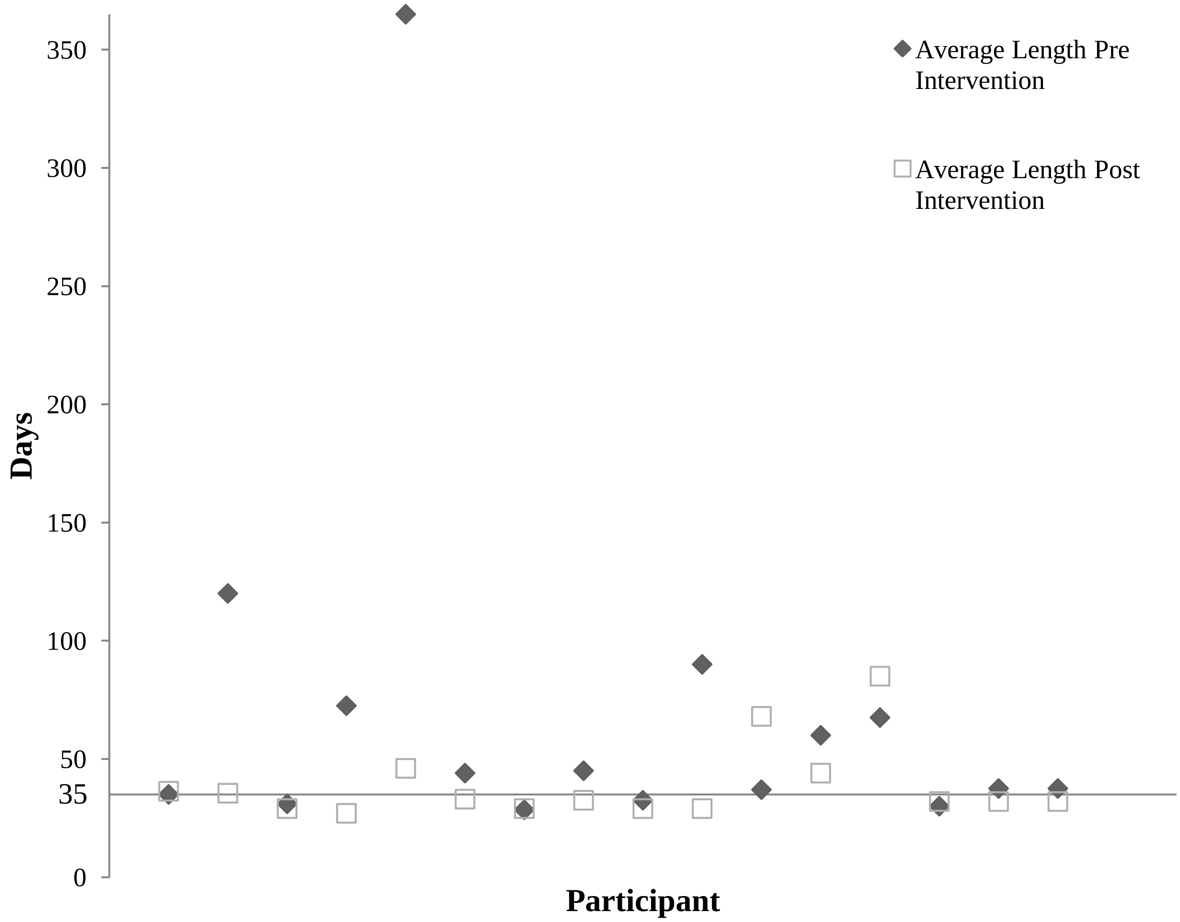

Figure 5.2. Comparison of mean intervals between menses in pooled study participants before and after the intervention. Data were analyzed using the Wilcoxon signed-rank test.

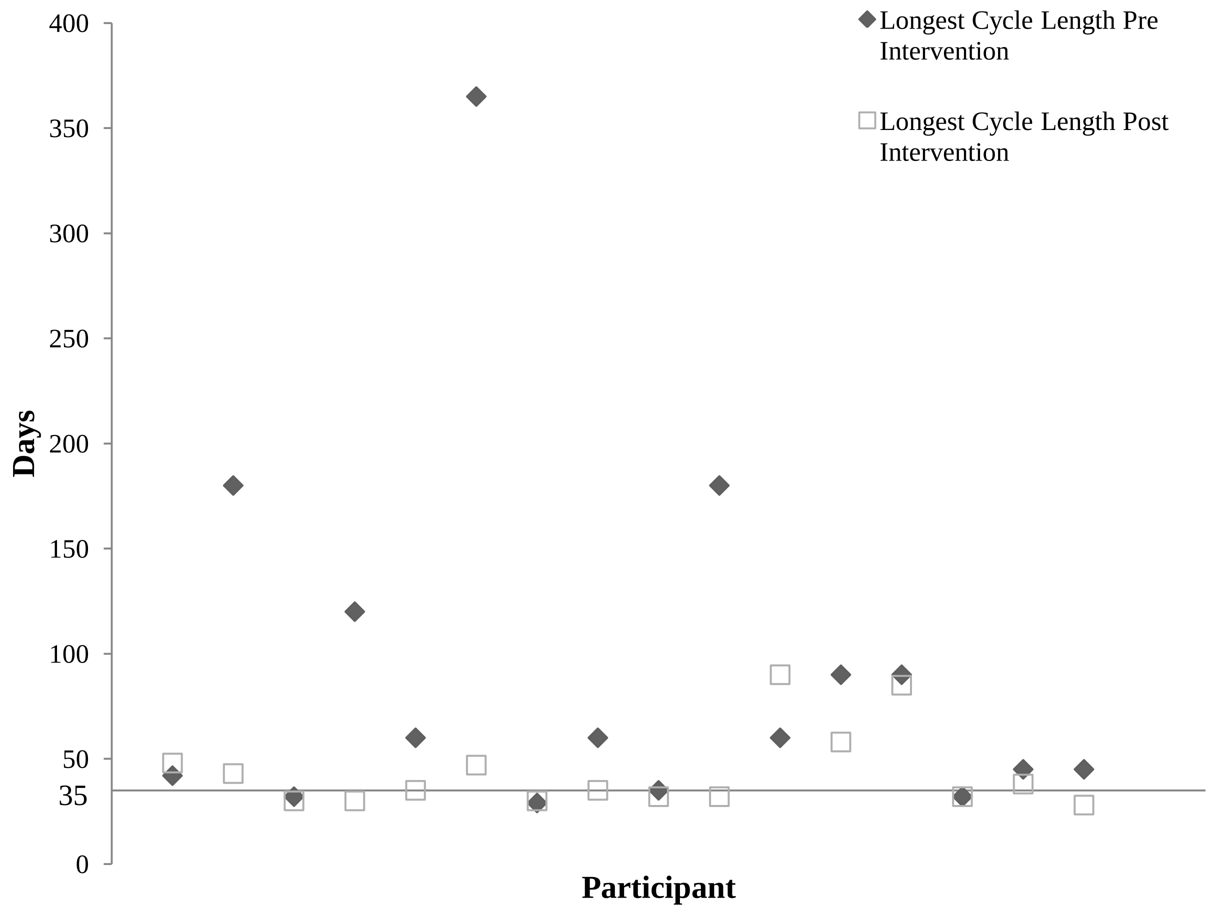

Figure 5.3. Comparison of longest intervals between menses in pooled study participants before and after the intervention. Data were analyzed using the Wilcoxon signed-rank test.

CHAPTER 6

DISCUSSION

6.1 General Discussion

Our objectives were to evaluate the effect of a pulse-based diet and exercise lifestyle intervention in women with PCOS. Women with PCOS are prone to insulin resistance (IR) and subsequent hyperinsulinemia which affects lipid metabolism, and androgen production. A subgroup of women with PCOS who have IR also go on to develop insufficient pancreatic β-cell failure resulting in hyperglycaemia and Type 2 diabetes mellitus (15).

We theorized that the diet and exercise intervention would result in a decrease of several attributes of the body composition of our participants. Specifically, we thought that weight, BMI, waist circumference, body fat percent, antral follicle count, fasting glucose insulin levels, HOMA score, abdominal adiposity and length between menstrual bleeds would be reduced on the pulse diet. Our hypothesis was supported with regards to fasting insulin levels and HOMA score. Although we observed a significant decrease in weight, body fat percent, AFC in the right ovary, fasting insulin levels, HOMA score, and a significant decrease in time between menses due to the lifestyle intervention, it was not due solely to the pulse-based diet. The participants' BMI, WC, AFC in the left ovary, and abdominal adiposity were not affected.

The results generated in the present study are consistent with the data found in previous combined dietary and exercise lifestyle interventions. The intervention in the present study did not encompass any calorie restriction but encouraged a low-GI diet. The majority of protein, carbohydrates and protein in the pulse arm came from pulse sources (chickpeas, peas, beans and lentils) and the TLC diet followed the National Cholesterol Education Program. Pulses, such as chickpeas, split peas, beans, and lentils are a form of a slower absorption carbohydrate that contributes to a lower glycemic index (GI) diet. It follows that pulses thereby help to prevent the onset and the impact of Type 2 diabetes mellitus (151). Marsh et al. (157) conducted a 12 month study looking at the effects of a low-GI diet as opposed to a high-fiber, high-GI diet in overweight and obese women with PCOS. They reported that women who were randomized to the low-GI diet arm had 3-fold greater improvement in whole-body insulin sensitivity as measured from responses to the oral glucose tolerance test. In addition, a larger proportion of women following the low-GI diet reported increased menstrual regularity. Both of these results are consistent with what we have observed in our intervention.

Numerous diet and exercise lifestyle interventions have been shown to have positive effects in terms of clinical and metabolic parameters in women with PCOS. Exercise interventions have reduced IR, improved body composition measures (such as weight, BMI, and WC), and increased menstrual cyclicity and ovulation. Combined dietary and exercise lifestyle interventions have been shown to improve anthropometric measurements, especially weight loss in addition to reduced IR and increased reproductive function (158). A recent systematic review and meta-analysis has demonstrated that lifestyle modifications in women with PCOS have been shown to have a small but significant effect on glucose and insulin blood levels. In addition, weight loss in the PCOS population was significantly and directly correlated with improvements in metabolic parameters, such as reduced IR (159).

It could be speculated that an intervention based purely on exercise would show a greater change in physical aspects such as WC and BMI. Women having a calorie restricted diet would be expected to have more of an effect on all anthropometric features. Since our study encompassed a change in diet with no calorie restriction in addition to exercise, it was reasonable to expect a modest amount of weight loss and subsequent resumption in menstrual cyclicity (5). It would also be reasonable to expect changes in insulin levels due to the consumption of a lower glycemic diet coupled with nutrition and health education in the PCOS population. Our participants exhibited significant decreases in weight, fasting insulin levels, and length in between menstrual cycles.

One diagnostic criterion for PCOS is polycystic ovaries on ultrasound examination. Polycystic ovaries contain more than 12 follicles measuring 2 to 9 mm following a characteristic peripheral 'string of pearls' distribution or a volume of >10mL as defined by the Rotterdam consensus in 2003. Dewailly et al. (49) and Lujan et al. (2) suggested increasing the threshold for the diagnostic criteria for PCO because newer ultrasound technologies and detailed post-hoc analyses of ultrasound images resulted in artificial inflation of women with PCOS using only the follicle numbers used in the Rotterdam consensus. Therefore, we used the diagnostic criteria of over 25 follicles to diagnose a polycystic ovary. We speculated that the intervention would create metabolic changes that were reflected in a decrease in follicle population.

We observed that antral follicle counts were significantly decreased in the right ovaries of the participants but not in the left ovaries. No difference in follicle number was detected between the pulse and TLC groups. Therefore, follicle numbers from the pulse and TLC groups were pooled to discern any trends in follicle number before and after the intervention. In the right ovary, 20 ovaries were able to be evaluated. During the study, it became apparent that the body size of some of the participants made the left ovaries difficult to examine. We were unable to demonstrate a decline in antral follicle count in the left ovaries of participants even though a trend of decreased follicle numbers was observed (38 to 32 follicles). It is possible that by increasing the participant number we may find a decline in follicle number following changes in diet and exercise. The reduction in follicle number over the intervention was indicative of a short term effect of diet and exercise changes in women with PCOS. It could be expected that follicle reductions would be observed in a greater capacity over an intervention that spanned a longer period of time or during follow-up appointments in participants who continued to follow diet and exercise modifications.

We were unable to locate any published research focused on the AFC of women with PCOS before and after a diet and/or exercise intervention. Most research trials were focused on resumption of ovulation implied by the onset of regular menses. It is difficult to say whether a reduction in the number of follicles detected in the ovary is a beneficial measure in women with PCOS. Follicle number on its own is not an indicative measure of reproductive health in women with PCOS. A participant with PCOS could demonstrate no change in follicle number after the intervention but could have started ovulating. Perhaps we should have focused on detecting improvements in the health of the follicles and the resumption of ovulation in addition to follicle number to gain a better understanding of the impact of diet and exercise changes in reproductive health measures in women with PCOS. However, we speculated that a reduction in follicle number may indicate that a smaller number of follicles remained in a static state. Typically, follicles that remain in a static state are induced by increased ovarian androgens (32). A decreased AFC could possibly be associated with decreasing ovarian hyperandrogenism, an improvement in the metabolic state of an individual (decreasing hyperinsulinemic environment) and a better follicular response to gonadotropins (39).

The decreasing hyperinsulinemic environment seen post intervention may have been a result of a significant decrease in fasting insulin levels. There was also a tendency toward a change in insulin levels between the pulse and TLC group. This observation has been consistent with previous findings in a variety of lifestyle intervention studies with PCOS. Insulin acts to regulate glucose homeostasis by stimulating glucose uptake in target tissues. Insulin also suppresses hepatic glucose production and lipolysis. Women with PCOS often present with higher than normal fasting insulin levels and IR.

We also observed a decrease in the HOMA score of participants before and after the intervention, with a tendency toward a difference due to the pulse based diet. It should be noted that our population was very insulin resistance with a mean HOMA score of 22.7 before the intervention and a mean HOMA score of 14.9 after the intervention. Clinically, the cut off for IR is 2.6 (78). Insulin resistance is often associated with increased fat storage, decreased fat breakdown and an increase in LH mediated androgen production in women with PCOS. Therefore, decreasing insulin levels and subsequently, insulin resistance in the PCOS population holds a variety of health benefits. A decrease in fasting insulin levels is related to decreasing the acquisition and impact of hyperinsulinemia and T2DM (83). Decreasing insulin resistance is highly associated with gaining reproductive function through decreasing androgens and improving the ovarian hormonal environment allowing maturation of follicles (90).

We did not detect a significant change in abdominal adiposity although a reduction in fasting insulin levels was observed post intervention. Abdominal adiposity was measured using whole body DEXA scanning. The most accurate technique for measuring abdominal adipose tissue *in vivo* is computed tomography (CT). CT scanning was not readily available because of its cost, limited access to equipment, and exposure to ionizing radiation that was not clinically justified. The DEXA method relies on the differential absorption of x-rays to distinguish different body tissues with minimal exposure to radiation and allows the direct measure of the quantity of fat present in different body regions can be obtained from DEXA imaging. DEXA scanning is a comparable and validated substitute to CT scanning and is sensitive to small changes in body composition (98, 99). We used DEXA scanning to measure abdominal adiposity before and after the intervention.

Abdominal adiposity is usually elevated in women with PCOS and affects between 50% to 60% of women with PCOS, despite matching for age and BMI (95). The development of abdominal adiposity in women with PCOS is multifactorial. There is a genetic basis for the increased abdominal adiposity with the most likely candidates being the genes involved in the regulation of ovarian steroidogenesis, BMI, and adiposity in addition to the effect of hyperandrogenism on the adipocyte. These factors may be exacerbated by an obesogenic environment (poor diet and exercise). Abdominal adiposity is often associated with IR. Hyperinsulinemia occurs as a consequence of IR and contributes to androgen excess. Visceral adiposity develops as a result of a hyperinsulinemic environment and hyperinsulinemia induce additional hyperinsulinemia and increased IR. Visceral adipose tissue may also contribute to hyperandrogenism using mechanisms independent from IR via secretion of inflammatory factors, adipokines, and local metabolism of sex steroids (83).

Abdominal adiposity as determined by DEXA is highly correlated with waist circumference. Women with PCOS often have a higher WC in parallel with higher abdominal adiposity. We did not observe a significant decrease in WC which was consistent with the abdominal adiposity measured obtained. It could be speculated the lack of caloric restriction in our diet and exercise intervention contributed to no decline in stationary WC. An exercise based intervention would be expected to have a greater effect on body composition and induce a decrease in WC and abominal adiposity. A hypocaloric diet intervention would be expected to result in a decreased body weight and BMI (116). Prior investigations have demonstrated that there are a variety of ways to improve insulin resistance to alter a hyperinsulinemic and hyperandrogenic profile can be applied to women with PCOS. A decrease in abdominal adiposity (and WC) is important for women with PCOS because it is correlated with decreasing IR and visceral adiposity (134).

Menses can be used as an external sign to mark an increase in fertility in addition to decreasing the risk of uterine cancer in this population (126). Resumption of cyclic menstrual bleeding patterns and possibly ovulation typically follows even a modest loss of weight (approximately 5% to 10%) (8). These findings are consistent with our results. We observed a decrease in the average length between menses as well as a decrease in the longest length between menstrual cycles after the intervention. Clinically, menstrual cycles are classified as regular (bleeding every 21 to 35 days) and irregular (bleeding >35 days). Regular menstrual bleeds demonstrate a cyclic pattern whereas irregular cycles are anovulatory and demonstrate no cyclicity. In most women, a decreased interval between bleeds translated into the resumption of menstrual cyclicity. Over duration of the intervention, we noticed a pattern of resumption of menstrual cycles that were not classified as regular but did have a cyclic pattern (33 to 50 days). This prolonged cycle pattern is not referenced in literature but can be observed clinically in participants with PCOS who undergo diet and exercise changes for when attempting to ameliorate infertility. The cyclic pattern indicates that ovulatory incidences probably occur with a possibly elongated follicular phase compared to previously longer intermenstrual intervals with no discernable pattern. Follow up studies in women with PCOS should be conducted to determine the length of the follicular phase and the incidence of ovulation post intervention.

Resumption of menstrual cyclicity could be used as a positive marker of the benefit of lifestyle interventions in conjunction with decreasing AFC. Even though AFC and menstrual cyclicity are related measures, they may also be used to infer different factors in the fertility and metabolic parameters in women with PCOS. It is difficult to ascertain whether reduction in follicle number alone is indicative of women with PCOS benefiting from diet and exercise interventions. Decreasing AFC is correlated with decreasing ovarian hyperandrogenism and a better follicular response to gonadotropins (97). Folliculogenesis is highly dependent on ovarian paracrine factors; only the last two weeks of antral follicle growth are dependent on circulating gonadotropins (26). Reduction in follicle number can then be attributed to a reduction in local ovarian hyperandrogenism and possibly a local reduction of a hyperinsulinemic environment. The local reduction coupled with a systemic reduction in insulin levels and correlated IR could synergistically lead to an increase in menstrual cyclicity without necessarily having a significant reduction in PCO morphology.

6.2 Study Limitations

There were several limitations encountered by the research team during the trial. Long-term adoption of diet and lifestyle modifications have been difficult to attain and proven efficacy of long term changes has not been realized. One of the most difficult aspects of diet and lifestyle interventions, especially in the PCOS population, is the high rate of non-compliance and dropout in lifestyle interventions. Our drop-out rate of 33% was consistent with previous studies conducted in women with PCOS and limited our observations to a small participant number (158). Although 49 women with PCOS were randomized into our intervention, only 25 women completed the intervention. Only 20 of the 25 women could be adequately examined ultrasonographically to evaluate for a change in AFC before and after the intervention. The ovaries could not be visualized clearly for AFC analysis because of abdominal adiposity, therefore there were fewer results for the study. A greater number of participants are needed to provide statistical power. Numerous investigations have recommended diet and lifestyle modifications to women with PCOS.

We found that consumption of the pulse diet was difficult for most participants as pulses were not part of their past diet. The accommodation to the pulse diet took some time. Frequent complaints about the pulse-based dietary intervention included poor flavour and texture (bland, unusual spices, lack of meat flavours), excess food volume and adverse gastrointestinal effects associated with the consumption (i.e., gas, nausea, fullness). Compliance with the study protocol was also affected by social factors, such as eating different foods at family meals, in restaurants, and during holidays. Exercise compliance was difficult to attain for most participants in the program. The recommended exercise (3 to 5 times a week) was difficult for many participants to achieve. Reasons for non-compliance in exercise included having a previously sedentary lifestyle (no gradual shift from nothing to full compliance) and issues with time (i.e., not being able to go to the gym because of night shift hours, family commitments and gym hours). We could not determine specific numbers for exercise compliance at this point in time in the intervention.

The blood samples taken during the intervention were analyzed in the clinical laboratory of the health region. Because of the expense involved with the analyses, we were limited to accepting a wider standard error that exists in health laboratories that analyze samples without a single control. There may be a benefit to re-analyze certain blood samples using assay techniques with standard controls used in research to generate increasingly precise metabolic and hormonal profiles to gain more accurate results for with more sensitive assays.

6.3 Future Directions and Clinical Applications

The preliminary results of participants who had finished the study are promising. The results are consistent with previous lifestyle intervention studies conducted in women with PCOS especially in terms of anthropometric features, menstrual cyclicity and fasting insulin levels. A logical next step would be to assess the impact of the intervention on other metabolic parameters, such as lipid profiles, HbA1c, and changes in markers of inflammation such as C-reactive protein (CRP). An decrease in the HDL:LDL ratio may indicate positive impact of the intervention on lipid levels and decreased risk of cardiovascular disease. Glycated hemoglobin (HbA1c) is measured to identify the average plasma glucose concentration over a prolonged period of time. A small decrease in HbA1c levels, coupled with decrease in fasting insulin levels, would strongly support a positive benefit of the intervention in long term glucose in women who do not have diabetes. A decrease in CRP would indicate decreased systemic inflammation which would be a positive effect of the intervention.

Behavioral data of the participants before and after the intervention have been collected and are available for further analysis using an online survey as part of the larger study. It will be valuable to assess education, knowledge, and attitudes in women with PCOS toward various aspects of PCOS before and after the intervention. Topics of interest including fertility, diabetes, body image, nutrition, and exercise will provide an insight into the psychological disposition of women with PCOS and the impact and benefit of education about PCOS on participants' overall well-being. In addition, accelerometer data has been collected to document participants' activity levels before and after the intervention, which will provide good insight into long-term acquisition and retention of activity (5). Six and 12 month follow-ups are ongoing to assess the long-term sustainability of the diet and exercise lifestyle intervention study. It is important to assess how well changes in diet and exercise can be maintained in women with PCOS as it is a lifestyle change that is necessary to prevent future health problems such as Type 2 diabetes mellitus and metabolic syndrome in the PCOS population.

6.4 General Conclusions

Polycystic ovary syndrome has a large heterogeneity in presentation and is one of the most common endocrine disorders occurring in women of a reproductive age. PCOS affects about 6% to 7% of the population (1). Women with PCOS are predisposed to developing obesity, Type 2 diabetes mellitus, cardiovascular disease, and uterine cancer and also report a decreased quality of life (15).

In the present study, we have learned that consuming food of a lower glycemic index (pulse crops) without a calorie restriction shows promise in helping women with PCOS gain healthier anthropometric profiles, decrease serum insulin levels and insulin resistance and increase their menstrual cyclicity. Arguably, early intervention by a multidiscplinary team of dieticians, exercise physiologists, and gynecologists would benefit women with PCOS and alleviate many of the physiologic and health concerns with PCOS.

Further study involving weight reduction and dietary intervention with pulse crops included as part of a regular diet may prove to be more successful than calorie reduction alone. Additional work regarding attitudes toward dietary interventions is needed to address how women with PCOS can include pulses in a diet that has been generally devoid of pulses. Pulse crops are a sustainable food resource that contain high levels of proteins and a slow absorbing carbohydrate component that lowers the GI of a diet. The acceptance of pulses is imperative to mediate positive health benefits in a long-term capacity with the increasing rise of T2DM, especially in the PCOS population.

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APPENDIX A

Manual of Operating Procedures for

A Lifestyle Intervention for Women with Polycystic Ovary Syndrome: The Role of a Pulse-Based Diet and Aerobic Exercise on Infertility Measures and Metabolic Syndrome Risk

Study #10-98

December 2010

Sponsor: Saskatchewan Pulse Growers and Agriculture – Agri Food Canada

TABLE OF CONTENTS: (Hold Ctrl + click to follow link)

APPENDICES (Hold Ctrl + click to follow link)

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ABBREVIATIONS

The following abbreviations are used throughout this document.

BP: blood pressure
NCEP National Cholesterol Education Program
PCOS: polycystic ovary syndrome
RA: research assistant
RD: registered dietitian
RUH: Royal University Hospital
TLC: Therapeutic Lifestyle Changes
w: week
WC: waist circumference

BACKGROUND

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, affecting approximately 105 million women worldwide (Azziz, 2005) and an estimated 1.4 million women in Canada (Lujan et al, 2008). Women with the syndrome present with several clinical signs, which include menstrual and ovulatory dysfunction, hyperandrogenemia (excess male sex hormones), hirsutism (male-pattern hair growth in women e.g. facial, chest, abdominal hair), polycystic ovaries, insulin resistance, and hyperinsulinism (Azziz et al., 2009). A diagnosis of PCOS is accompanied with an increased risk of severe and devastating consequences such as infertility, dysfunctional uterine bleeding, endometrial carcinoma, obesity, type 2 diabetes mellitus, dyslipidemia (alterations in blood lipids), hypertension, and possibly cardiovascular disease (Azziz, et al., 2009; Ehrmann, 2005; Liepa, 2008; Lujan et al, 2008).

The pathogenesis of PCOS is not fully understood; however, insulin resistance and associated hyperinsulinemia are believed to be the key contributing factors (Douglas, 2006; Moran & Norman, 2004). Insulin resistance and the subsequent production of excessive amounts of insulin are directly associated with the increased ovarian production of androgens. Increased androgen levels impede ovarian follicle growth, inhibit ovulation and normal menstruation, lead to the polycystic ovarian morphology, and generate male pattern hair growth seen in PCOS (Blank, McCartney, & Marshall, 2006). Between 50% to 70% of women with PCOS exhibit insulin resistance..

Metabolic complications of insulin resistance are higher among women with PCOS than the general population and include the development of dyslipidemia (i.e. increased cholesterol levels), type 2 diabetes mellitus, metabolic syndrome and increased risk for cardiovascular disease (Azziz et al., 2009; Ehrmann, 2005) (Apridonidze, Essah, Iuorno, & Nestler, 2005).. Metabolic syndrome is a clinical diagnosis for individuals who exhibit insulin resistance, hypertension, abnormal serum lipid levels (i.e. high triglycerides and low high-density lipoproteins) and abdominal obesity (Liepa, 2008). Serum androgen levels are higher in women with PCOS who develop the metabolic syndrome when compared with absence of metabolic syndrome. Women with PCOS who are obese present with greater insulin resistance / hyperinsulinemia, anovulation, abnormal uterine bleeding and tend to be at greater risk for early development of metabolic and neoplastic complications (Lau, 2007). Many of the medical therapies for PCOS are less effective in women who are obese (Palomba, 2008).

Lifestyle modifications directed at reducing insulin resistance, including diet and exercise, are essential for effective management of PCOS. Little is known about the insulin lowering effect on the polycystic ovarian morphology. Most therapies are directed at short-term goals, to induce ovulation and restore fertility or to ameliorate hirsutism or abnormal bleeding. Exercise and dietary lifestyle changes are recommended as the first line of therapy to restore ovulation. Because lifestyle changes are often difficult to achieve, medical therapy is used to induce ovulation. Long term therapeutic management needs to be implemented to control obesity, hirsutism, irregular menstruation and infertility and to prevent metabolic syndrome, diabetes, heart disease and cancer that afflict this vulnerable group of women (Marsh, 2005).

The proposed study will examine a pulse-based diet as a nutrition intervention for the PCOS population. The rationale for this intervention is supported by the association of pulse-based diets with improved glycemic control (Sievenpiper et al. 2009). The use of a pulse-base diet is further supported by data originating from our research group which has shown positive effects of including pulses in the diet of individuals at risk for metabolic syndrome.

PURPOSE

The purpose of this study is to evaluate a lifestyle intervention for women with PCOS.

OBJECTIVES

There are five main objectives of the proposed study:

1. To characterize the lifestyles (i.e. diet including pulse consumption and exercise), quality of life, other health indicators for risk of disease (e.g. blood parameters, anthropometry, body composition) menstrual history and fertility in a large sample (n>150) of women with PCOS (from baseline measures)

2. To determine the short-term therapeutic effects of a pulse-based diet on multiple disease measures of PCOS, metabolic syndrome, and quality of life after 16 weeks.
3. To determine the long-term therapeutic effects of a pulse-based diet on the multiple disease measures of PCOS, metabolic syndrome, and quality of life after the 16 week therapeutic intervention through follow-up measurements of the participants (at 6 months and 12 months post-intervention)
4. To measure the rate of long-term adoption of a pulse-based diet during the 6-12 months following the lifestyle intervention.
5. To measure the effect of a pulse-based diet intervention on participants' consumption of pulse foods after completion of the intervention diet.

HYPOTHESES

1. Introduction of a pulse-based diet for 16 weeks will reduce hyperinsulinemia and improve other markers of the metabolic syndrome (i.e. reduce glucose, triglycerides, blood pressure, abdominal fat, and increase HDL); and therefore reduce the disease measures, symptoms of PCOS (i.e., increased number of ovarian follicles, increase levels of estradiol and progesterone and decrease androgens).
2. Women with PCOS who are given a 16 week pulse-based diet will continue practicing the consumption of pulses for 6 and 12 months after the intervention and this will continue to improve their resting insulin levels, markers of metabolic syndrome, and symptoms of PCOS.
3. The pulse-based diet will increase the quality of life of women with PCOS.

DESIGN

8.1 See Appendix A Research Participant Information Sheet and Consent Form and Appendix B for the Researcher's Summary Form.

8.2 The study is a single-blind parallel stratified-randomized design. Randomization will be stratified based on medication use (i.e. medications for PCOS symptoms, glucose control, lipid-lowering, or hypertension and those who are not taking medications).

8.2.1. Participants will be randomly assigned to one of two diet programs:
1) Participants in Program 1 will receive the placebo diet (n= 84)
2) Participants in Program 2 will receive the pulse-based diet (n= 84).

8.2.2. All participants will also receive supervised aerobic exercise training to provide recommended standard of care and to reduce variability in this lifestyle factor between participants. To control for diet variability, all participants will be instructed to follow the dietary recommendations of the Therapeutic Lifestyle Changes (TLC) guidelines developed by the National Cholesterol Education Program (NCEP) Expert Panel on

Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, 2002).

Table 1: TLC Guidelines: Recommended Nutrient Composition

Saturated fat less than 7% of total calories
Trans fatty acids are another LDL-raising fat that should be kept at a low intake.
Polyunsaturated fat up to 10% of total calories
Monounsaturated fat up to 20% of total calories
Total fat 25-35% of total calories
Carbohydrate 50-60% of total calories (carbohydrate should be derived predominantly from foods rich in complex carbohydrates including grains, especially whole grains, fruits, and vegetables)
Fibre 20-30 g/day
Protein approximately 15% of total calories

DIET PROGRAMS

* A separate manual exists for specific dietary guidelines*

9.1 Program 1: Placebo Diet

9.1.1 Participants assigned to Program 1 (placebo) will be asked to follow the Therapeutic Lifestyle Changes (TLC) diet. Participants will be asked to attend a 90-minute long diet education session, where a Registered Dietitian will explain the TLC guidelines.

9.1.2 The first two weeks of the program are referred to as the “lead-in period” (the period of time where all participants will follow the TLC diet). Following the “lead-in period” participants in Program 1 will be asked to continue the TLC diet for 16 weeks. Two meals will be supplied daily for 16 weeks to those participants in the placebo group. Meals will follow TLC guidelines and will be based on lean-meats for the protein source. The meals will exclude pulses.

9.2 Program 2: Pulse-Based Diet

Participants assigned to Program 2 will be asked follow the TLC diet and will receive a pulse-based diet. Participants will be asked to attend a 90-minute long education session, where a Registered Dietitian (RD) will explain the TLC diet guidelines. Participants will follow the TLC diet during the lead-in period and will be asked to follow the TLC diet for 16 months in addition to consuming a pulse-based diet. The pulse based-diet will include meals prepared with dry peas, lentils, chickpeas, and beans. Two meals will be supplied daily for 16 weeks to those participants on the pulse-based diet program. Meals will contain approximately 90 g dried green peas, 225 g chickpeas or fava beans, or 150 g lentils. The length of this intervention was based on a review of pulse-based diets that improve insulin and glucose levels (Sievenpiper et al., 2009).

EXERCISE PROGRAM

All participants (Program 1 and Program 2) will follow an exercise program for the 16 weeks of the study. The exercise program involves 5 sessions of aerobic exercise per week: 3 sessions of supervised exercise training and 2 sessions of unsupervised at-home exercise. The supervised exercise training will occur in the College of Kinesiology at the Williams Building (treadmill walking, exercising on a rowing machine, elliptical trainer, or exercise bike). Each exercise session will be 45 minutes long. This amount of aerobic exercise is the minimal level of exercise shown to be beneficial for increasing high-density lipoproteins and lowering triglyceride levels (Durstine, 2002). We also have an exercise training laboratory that is fully equipped with all the required exercise machines and reserved exclusively for research participants. Participants will be encouraged to exercise at an intensity of at least 60% of their age-predicted maximal heart rate (i.e. 220-age).

SELECTION AND WITHDRAWAL OF PARTICIPANTS

11.1 Inclusion Criteria

Women between the ages of 18-35 years who have a diagnosis of PCOS. To daignoseFor a diagnosis of PCOS, two of three criteria must be present:

1) irregular or absent menstrual cycles,
2) high blood level of male hormone or male pattern hair growth, and
3) polycystic ovaries (enlarged ovaries with multiple small follicles (egg sacs) in the ovary) confirmed by transvaginal ultrasound examination.

11.2 Exclusion Criteria

Women are excluded from the study if they:

11.2.1 Are using hormonal birth control or fertility medications, antiseizure or antipsychotic medications known to induce the development of polycystic ovaries or hyperprolactinemia

11.2.2 Have a medical condition that limit their ability to exercise to 60% of their maximal heart rate

11.2.3 Cannot consume a pulse-based diet (allergies or intolerances).

11.3.4 Hhave an uncontrolled medical condition that interferes with ovarian or systemic hormone production

11.3 Participant Withdrawal Criteria

11.3.1 A participant who develops any of the above exclusion criteria will be withdrawn from the study by the principal investigator.

11.3.2 If an adverse event involving a participant is mild or moderate, the participant will still be allowed to continue in the program if there is no contraindication following medical assessment. but may also withdraw if she chooses.
11.3.3 See Appendix C for Adverse Events Forms.
11.3.4 Withdrawn participants are not to be replaced.

11.4 Discontinuation Criteria for Individual Participants and for the Trial
Because we are including an "intent-to-treat" analysis, participants who are not compliant with the pulse diet or exercise training will not be excluded from analysis.

SAMPLE SIZE

12.1 A recruitment target of 166 women (n=83 per group). However, to facilitate randomization, a new recruitment goal of 168 has been established (n=84 per group).

12.2 Participants will be staggered into the intervention over a year so that an equal number of participants are assessed across the different seasons (winter, spring, summer, and fall). This is to account for the known seasonal variation in blood lipid variables (Ockene et al., 2004). Every three months for a year, 42 women (21 for each Program 1 and 2) will enter the study (8 groups of 21 women) for a total of 168 women. See Table 2.

12.3 Table 2: Study Groups

	Placebo Diet	**Pulse-Based Diet**
Season 1	21	21
Season 2	21	21
Season 3	21	21
Season 4	21	21

12.4 The sample size calculation was based on the measurement of fasting insulin levels. A recent meta-analysis conducted by Sievenpiper et al. (2009) showed an effect size for pulse diets on insulin as -0.49. Women with PCOS have a fasting insulin level of approximately15 uU/mL and a SD of 9 uU/mL (Macut et al. 2008; Glueck et al. 2009). An effect size of -0.49 with a pulse-based diet would result in end-of-treatment insulin level of about 10 uU/mL. To achieve a power of 80% and alpha of 0.05, 66 participants per group are required. This is a long-term study; therefore, we anticipate that there may be participant drop-outs.

12.5In previous studies by the researchers, a drop-out rate of __% has been noted in addition to participants not completing all elements of the study. To account for this we are enrolling 84 participants per group to achieve at least 66 participants for analysis.

RECRUITMENT

13.1 Status of Clinical Trial

When recruitment officially begins, the kinesiology RAs will contact Lindsay Tumback to update the status of the trial to "recruiting" on ClinicalTrials.gov.

13.2 Methods of Recruitment

13.2.1 Women will be recruited through posters and online advertisements (e.g. PAWS), Kijiji, throughout the University of Saskatchewan, SIAST, physician offices, health care establishments, and advertisements in local newspapers. First, inexpensive forms of advertizing will be used (e.g. posters, online). If deemed necessary, Dr. Chilibeck will arrange for the advertisement to be posted in the newspapers.

13.2.2 See Appendix D for the recruitment poster. Smaller pieces of paper with the study name and contact information should be printed to accompany the posters (for interested women to take home with them). The kinesiology RAs will speak to Dr. Chizen and Dr. Pierson regarding where to display the posters (based on their experience with previous PCOS studies).

13.3 Inquiries from Interested Women

13.3.1 Kinesiology RAs will be answering inquiries from women who respond to the advertisements. The kinesiology RAs will provide general information regarding the study and will go over the Inclusion and Exclusion Checklist (Appendix E). The Inclusion and Exclusion Checklist will determine if the women might possibly have PCOS. The kinesiology RA will explain that the diagnosis of PCOS will need to be confirmed by a medical appointment with Dr. Chizen.

13.3.2 If eligible to participate, a copy of the consent form will be forwarded to the potential volunteer by email or Canada Post mail. The volunteer will meet with a Kinesiology RA or with Dr Chizen, Dr Pierson or Shanni Serrao to review and sign the consent form.

13.4 Booking Medical Evaluation

13.4.1 Once consent has been obtained, the kinesiology RAs will:
-assign a participant code
-schedule each potential recruit to attend a medical evaluation with Dr. Chizen during a PCOS Research Clinic time to confirm the presence of PCOS. (See 14.1 below)

13.4.2 To schedule the appointment with Dr. Chizen, the kinesiology RA's will contact Shanni Serrao at the.Women's Health Imaging Research Laboratory (WHIRL); alternately, contact Ms Heather Schultz, Dr. Chizen's office assistant

PCOS RESEARCH CLINIC TIMES AND ENROLMENT

14.1 Establishing the PCOS Clinic Times

14.1.1 As of March 2011 Dr Chizen will make time available on Thursday all day and Tuesday before 1030 am; alternately Friday afternoon after am appointment and ultrasound appointments.

14.1.2 Shani Serrao (966 7873- Women's Health Imaging Reproductive Laboratory (WHIRL) grad office) will coordinate appointment scheduling with Dr Chizen;

14.1.3 Alternately, Dr Chizen's office assistant, Heather Schultz will arrange appointments (966 8623).

14.1.4 If alternate clinic appointment times are needed, appointment times will be arranged in consultation with Dr Chizen.

14.2 Researchers Present for PCOS Clinic Times

Dr Chizen and Shani Serrao. Dr Pierson may also perform ultrasound examinations.

14.3 Initial Appointment

14.3.1 Dr. Chizen will complete a Medical Assessment Form (Appendix F).

14.3.2 Dr Chizen will perform a transvaginal ultrasound examination. Following appropriate training, Shani Serrao may perform the transvaginal ultrasound examination.

14.3.3 Dr. Chizen will provide a requisition for screening blood work if a preliminary diagnosis of PCOS is made

14.3.4 The potential recruit may attend any SHR lab for the screening bloodwork

14.4 Follow-up Appointment

14.4.1 Scheduling the Follow-Up Appointment

14.4.1.1 All women who attend the initial appointment will receive a call from Dr. Chizen's office (Shani Serrao) when their blood analysis results are available to schedule a follow-up appointment. (Alternately, Heather Schultz may schedule an appointment).

14.4.1.1.1 The follow-up appointment will be booked between day 1-5 of menses for women with regular cycles.

14.4.1.1.2 The follow-up appointment may be booked without regard to a menstrual cycle dates for women who have unpredictable menstrual cycles.

14.4.1.1.3 This appointment will occur during the 2 week lead-in period.

14.4.1.2 The kinesiology RA will be contacted by Shani / Heather/ Dr Chizen to begin the enrollment procedure to coincide with the period of performance of 2 hour fasting blood tests/repeat ultrasound.

14.4.2 Follow-up Appointment Activities

At this appointment, if a diagnosis of PCOS is confirmed, Dr. Chizen (with Shani Serrao) will:

14.4.2.1 Execute the fasting blood tests and 2hr GTT blood tests in her office, and transport the blood samples to the RUH chemistry lab for separation and freezing. (see details, section 18)

14.4.2.2 Review the results of the participant's PCOS diagnosis blood tests with the participant, between the blood draws.

14.4.2.3 Complete the 2nd ultrasound between the blood draws. The second ultrasound examination will be completed during the 2 week lead in period, prior to the diet/exercise intervention

14.4.2.4 Confirm that the participant has seen or has an appointment with the kinesiology RA to begin the enrolment procedure

14.4.2.5 Women who do not have PCOS will be informed of the results and be advised about suitable follow-up elsewhere as indicated by their examination and blood samples.

14.5 Enrolment Procedure

14.5.1 Accelerometers are used at the beginning of the study to measure the participants' usual activity (i.e., before exercise program). Accelerometers are not used for activity monitoring during the program. More information on the use of accelerometers is found in the efficacy data section.

14.5.2 Once a diagnosis of PCOS has been confirmed, the participant will have been informed by Dr. Chizen to contact the kinesiology RA to being the enrolment procedure.

14.5.3 At this contact, the kinesiology RA will book a time for the participant to come in to the Williams Building Gym, where the kinesiology RA will follow the enrollment procedure:

14.5.3.1 The RA will provide a binder for the participant (to keep all the instructions, appointment bookings, etc.)

14.5.3.2 The participant will fill out the Subject Information Form (Appendix G)

14.5.3.3 The RA will administer the PAR-Q (Appendix H).

14.5.3.3.1 Certain Par-Q responses may require permission from Dr. Chizen or a family physician to start the exercise program (e.g., if on blood pressure medication, etc.).

14.5.3.4 RA will administer the Leisure-Time Exercise Questionnaire (see Appendix I)

14.5.3.5 RA will provide the participant with a package containing:

14.5.3.5.1 A 4-day food record (and review the food record with the participant) (see Appendix J)

14.5.3.5.2 Instructions for accessing the online Quality of Life Questionnaires or, if the participant doesn't have internet access, will provide paper copies and a self-addressed postage paid envelope

14.5.4 The RA will book the participant to attend the TLC guidelines presentation

14.5.5 The RA will provide the accelerometer and instructions on how to use the accelerometer (Appendix K)

14.5.6 The RA will schedule the first DEXA scan and provide instructions on how to find the DEXA office

14.5.7 The RA will take the physical measurements described in the efficacy data section

14.6 Follow-up Email

14.6.1 The kinesiology RA will send a reminder email to the participant (or phone call if no email access) approximately 3 days following enrolment.

14.6.2 The reminder email will remind participants to:

- contact the RAs at any time with questions
- fill out the 4-day food record
- access online questionnaires
- the date of the TLC presentation
- return accelerometers at TLC presentation

14.7 Subsequent Appointments at the PCOS Clinic

The kinesiology RAs will book an early a.m. appointment for the participants with Dr. Chizen(see section 14.1) at the end of their intervention (within the last 2 weeks). At this appointment, Dr Chizen will repeat fasting blood tests including a 2hr GTT, complete an ultrasound examination and review the menstrual history. The history and examination will be scheduled between blood draws.

TLC GUIDELINES PRESENTATION AND LEAD-IN PERIOD

15.1 The TLC Guidelines Presentation

15.1.1 To control for diet variability, all participants will be instructed to follow the dietary recommendations of the Therapeutic Lifestyle Changes (TLC) guidelines developed by the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, 2002).

15.1.2 Participants will be asked to attend a 90-minute long diet education session, to occur approximately a week after recruitment, where a Registered Dietitian (the nutrition RA) will explain the TLC guidelines. Handouts will be given to provide tips on healthy meals (developed by the nutrition RA) and healthy snacks, beverages and breakfasts (developed by nutrition students as professional practice projects).

15.1.2.1 At the TLC presentation, the nutrition RA will provide a package containing two 1-day food records

15.1.3 One kinesiology RAs will be present at the presentation to:

15.1.3.1 Collect the accelerometers (which will contain activity data that has been collected since enrolment -section 14.5.5).

15.1.3.2 Give two Leisure-time Exercise Questionnaires

15.2 The Lead-In Period

15.2.1 The first two weeks of the program are referred to as the "lead-in period" (the period of time where all participants follow the TLC diet). The lead-in period begins as soon as the participant has attended the TLC presentation. The purpose of the lead-in period is to standardize the placebo and study groups.

15.2.2 The exercise program and meal program do not begin until the lead-in period has finished.

15.2.3 During the lead-in period, the kinesiology RAs will contact the participant to book exercise times (which will begin immediately after the lead-in period).

HARD-COPY PARTICIPANT FILE

Each participant will have a hard-copy file with all data entered onto paper. This serves as a hard-copy back-up for the data entered onto the database. The hard-copy files will be located in a locked filing cabinet in the William's Building Gym office. The kinesiology RAs are responsible for maintaining the hard-copy files.

TIMELINES

Table 3 provides a general overview of the phases and main activities of the study. Table 4 details when each measure occurs during the phases.

Table 3: Timeline of Phases and Activities

Timeline of Phases and Activities

Phase	**0: Enrolment**		**1: Lead-In**		**2: Sixteen-week program**	**3: Follow-up**	
Week #	**1**	**2**	**3**	**4**	**5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20**	**6-months after the end of w20**	**12-months after the end of w20**
Activities	Enrolment Accelerometers worn starting at enrolment for 9 days The TLC presentation will occur at the end of this phase (end of w2) Participants will return accelerometers at the TLC presentation		All participants follow the TLC guidelines for two weeks No meals provided No exercise program		During these sixteen weeks: All participants continue to follow the TLC guidelines Meals will be provided for placebo and treatment groups All participants will follow the exercise program	No diet or exercise program	No diet or exercise program Accelerometers will be worn for 9 days

Key:
w: week

Table 4: Timeline of Measures

This table responds to Dr. Chilibeck's comments on original timeline (i.e. requiring more information on when measures will be taken during each phase). I think it is important to review, as your thoughts on the timing and frequency of the measures may change now that you see the table in this format (more detailed). Make take wt, BP, WG more often?

Note: change to original protocol: the lead-in ultrasound and DEXA were eliminated as they were deemed as unnecessary participant burden.

Timeline of Measures

Phase	**0: Enrolment**		**1: Lead-In**		**2: Sixteen-week program**																**3: Follow-up**	
Week #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**6-months after end of w20**	**12-months after end of w20**
Ultrasounds	**S**																			**E**	**S**	**S**
DEXA	**One measure once during these four weeks**																			**E**	**S**	**S**
BP and WC	**S**																			**E**	**S**	**S**
Weight and height	**S**																			**E**	**S**	**S**
Accelerometers	**S**			*****																		**S**
Par-Q	**S**																					
Leisure Time Questionnaire	**S**		**E**	**E**				**E**				**E**				**E**				**E**	**S**	**S**
1-day Food Record			**E**	**E**				**E**				**E**				**E**				**E**		
4-day Food Record	**S**																				**S**	**S**
Pulse	**S**																			**E**	**S**	**S**

Consumption Questionnaire						
Other Questionnaires	**S**			**E**	**S**	**S**
Routine PCOS Blood Measures	**S**	**E**	**E**	**E**	**S**	**S**
Routine Metabolic Syndrome Blood Measures	**S**	**E**	**E**	**E**	**S**	**S**
Two-hour Blood Measures	**S**	**E**	**E**	**E**	**S**	**S**
Saved Blood Measures	**S**	**E**	**E**	**E**	**S**	**S**

Key:
E: end of week
S: start of week
w: week
*ONLY if there is a lag between recruitment and when the participant actually starts the program, e.g., if the participant is recruited in one season, but starts in the next season

EFFICACY DATA

Note: for the following section, w# represents week number (e.g., w4 is week 4).

18.1 Imaging Measures

a) Transvaginal Ultrasounds	
Specific measures	Ovarian and uterine morphology (i.e., number and distribution pattern of ovarian follicles, endometrial thickness and pattern).
Where measures are taken?	PCOS clinic.
Who takes the measures?	Dr. Chizen and Dr. Pierson or trained ultrasound technician.
Where does the analysis take place?	Dr. Pierson's laboratory.
Appointment booking	The first ultrasound will occur at the initial visit with Dr. Chizen. See section 14.1, 14.2, 14.4 for booking information and appointments.
When measures are taken?	Once at initial visit with Dr. Chizen (pre-diagnosis of PCOS) Once at visit for fasting and 2 hr blood tests (w). Once at the end of the 16-week program (w18-20). Once at 6-month follow-up. Once at 12-month follow-up.
Applicable appendices:	None.

b) DEXA Reports	
Specific measures	Whole body fat mass. Abdominal fat mass (trunk fat). Whole body lean tissue mass.
Where measures are taken?	DEXA office in the William's Building.
Who takes the measures?	DEXA technicians.
Where does the analysis take place?	Dr. Chilibeck's laboratory.
Appointment booking	Kinesiology RAs will book DEXA scans with the DEXA technicians.
When measures are taken?	Once during phase 0 (between w1 and w4). Once at the end of the 16-week program (w20). Once at 6-months follow-up. Once at 12-months follow-up.
Applicable appendices:	None.

18.2 Physical Measures

a) Metabolic Syndrome Physical Measures	
Specific measures	Blood pressure (BP), weight, height, and waist circumference.
Where measures are taken?	William's Building Gym.

Who takes the measures?	Kinesiology RAs.
Where does the analysis take place?	To be determined.
Appointment booking	Measures will be taken by kinesiology RAs.
When measures are taken?	Once at initial visit with Dr. Chizen (start of w1). Once at the end of the lead-in period (end of w4). Once at the end of the 16-week program (18-20 week interval). Once at 6-month follow-up. Once at 12-month follow-up.
Applicable appendices	Revised Protocol for Measurement of Waist Circumference

18.3 Activity measures

a) Accelerometers (activity monitors)	
Specific measures	Activity will be measured using the Actical, a uniaxial accelerometer that detects vertical acceleration. Accelerometers are used at the beginning of the study to measure the participants' usual activity (i.e., before exercise program). Accelerometers are not used for activity monitoring during the program. The Actical (size and dimensions of a small pager) is worn by the participants on a belt worn around the waist with the accelerometer situated at the hip. The participants will be asked to record the time the monitor was attached and removed for the purpose of calculating activity time and sleeping time. The data are electronically downloaded into a data file that contains minute-by-minute movement counts for each participant. Participants will be wearing the monitor for 7 full days; deployed on day 1 and collected on day 9 (or at the TLC presentation). Because of the different times of deployment, participants will be asked to start wearing the accelerometer on day 1 (anytime) but data will only be collected from midnight on day 2 and then they wear it past midnight on day 7, then the device removed on day 8.
Where measures are taken?	The participants will be asked to wear the monitor at all times while awake. Exceptions would include water activities like bathing and swimming, or when it is deemed inappropriate by the participant.
Who takes the measures?	Participant wears monitor.
Where does the analysis take place?	Dr. Sherar will analyze the accelerometer data and will provide reports on participant activity.
Appointment booking	At enrolment, kinesiology RAs will loan the accelerometers to the participants. A log will be kept by the kinesiology RAs to record which accelerometers are loaned to which participants. The kinesiology RAs will teach the participants how to use

	the accelerometers at enrolment. The kinesiology RAs will instruct participants to return the accelerometers at the TLC Guidelines presentation.
When measures are taken?	Once at enrolment (w1). *Once at the end of the lead-in period (w4) (*ONLY if there is a lag between recruitment and when the participant actually starts the program, e.g., if the participant is recruited in one season, but starts the study in the next season). Once at 12-months follow-up.
Applicable appendices	Appendix K: Accelerometer Log.

b) Par-Q	
Specific measures	Participants will be asked to complete short questionnaires called the Par-Q (Canadian Society for Exercise Physiology, 2002).
Where measures are taken?	PCOS clinic.
Who takes the measures?	Participant fills out the questionnaire at enrolment.
Where does the analysis take place?	To be determined.
Appointment booking	Kinesiology RA to administer the questionnaire.
When measures are taken?	Once at the enrolment (w1).
Applicable appendices	Appendix H: Par-Q.

c) Leisure-Time Exercise Questionnaire	
Specific measures	Participants will be asked to complete the “Leisure-Time Exercise Questionnaire” to assess physical activity (Godin & Shephard, 1985).
Where measures are taken?	William’s Building Gym.
Who takes the measures?	Kinesiology RAs administer.
Where does the analysis take place?	To be determined.
Appointment booking	Kinesiology RAs will administer the questionnaire to participant before an exercise session.
When measures are taken?	Once at enrolment (start of w1). Twice during the lead-in period (end of w3 and end of w4). Four times during the 16-week program (end of w8, end of w12, end of w16, and end of w20). Once at 6-month follow-up. Once at 12-month follow-up.
Applicable appendices	Appendix I: Leisure-Time Exercise Questionnaire.

d) Exercise Compliance	
Specific measures	Participants will be asked to complete the Aerobic Exercise Tracking Logs

Where measures are taken?	William's Building Gym (supervised exercise) and at home (unsupervised exercise)
Who takes the measures?	Participant fills in form about their perceived compliance to the supervised exercise.
Where does the analysis take place?	To be determined.
Appointment booking	Not applicable.
When measures are taken?	Daily during exercise program.
Applicable appendices	L: Aerobic Exercise Tracking Logs

18.4 Dietary Assessment

a) Food Records	
Specific measures	To assess diet, participants will be asked to provide food records (records of what they have eaten in a day). These are diaries to filled out by the participant throughout the day. Note: these are <u>not</u> 24-hr recalls. Because the participant is recording what they are eating as the day progresses, they will likely change their eating habits to please the investigators. This limitation is acknowledged.
Where measures are taken?	Participants complete the records at home.
Who takes the measures?	Participants complete the records.
Where does the analysis take place?	Dr. Zello's laboratory (food records will be entered into Food Processor by nutrition RAs).
Appointment booking	Kinesiology RAs will provide the paper copies of the food records at the appropriate times. Note: the nutrition RA will give the lead-in food records to the participants at the TLC presentation.
When measures are taken?	**1-day food record:** Once at the start of w1 (at enrolment, PCOS clinic) Once at 6-month follow-up Once at 12-month follow-up **4-day food record:** Twice during lead-in (at the end of w3 and at the end of w4). Four times during the 16-week program (at the end of w8, end of w12, end of w16, and end of w20)
Applicable appendices	Appendix J: Food Record Note: the 4-day food record is the same form as the 1-day food record (make four copies of 1-day food record)

b) Pulse Consumption	
Specific measures	The Pulse Consumption Questionnaire will be administered to determine how often participants eat pulse foods.
Where measures are taken?	Participant completes the questionnaire online (or paper copy can be made available if no internet access).

Who takes the measures?	Participant completes the questionnaire online.
Where does the analysis take place?	Nutrition.
Appointment booking	Kinesiology RA sends email requesting participant to fill out questionnaire.
When measures are taken?	Once at enrolment (start of w1). Once at the end of the 16-week program (end of w20). Once at 6-month follow-up. Once at 12-month follow-up.
Applicable appendices	Appendix M: Instructions for Online Questionnaires Website.

18.5 Other Questionnaires

a) Quality of Life Questionnaires	
Specific measures	Two quality of life questionnaires and a brief motivational questionnaire will be administered (Wallston, Rothman, & Cherrington, 2007; Cronin et al., 1998; Colwell et a., 2010). The questionnaires will be administered via a secured website.
Where measures are taken?	Participants complete the questionnaires online (or paper copy can be made available if no internet access).
Who takes the measures?	Participant completes the questionnaire online.
Where does the analysis take place?	Psychology.
Appointment booking	Instructions for accessing the online questionnaires will be given by the kinesiology RAs at enrolment. The kinesiology RAs will send an email that requests the participant to fill out questionnaire.
When measures are taken?	Once enrolment (w1). Once at the end of the 16-week program (w20). Once at 6-month follow-up. Once at 12-month follow-up.
Applicable appendices	Appendix M: Instructions for Online Questionnaires.

18.6 Blood Measures

This section has been divided into five categories. Please read each category carefully:

a) Diagnosis of PCOS Blood Measures: Analyzed at the Saskatoon Health Region Laboratory
b) Routine PCOS Blood Measures: Analyzed at the Saskatoon Health Region Laboratory
c) Special Blood Measures That Will *Not* Be Measured at the Saskatoon Health Region Laboratory
d) Routine Metabolic Syndrome Blood Measures
e) Two-hour Blood Measures
f) Saved Blood Measures

a) Diagnosis of PCOS Blood Measures: Analyzed at Saskatoon Health Region Laboratory

Specific measures	These blood for these measures will be requisitioned as clinical care tests and participants will attend any of the SDH laboratories for blood draws; tests will be analyzed by the Saskatoon Health Region Laboratory as part of routine medical care (results are considered imprecise for research purposes). These measures include: CBC (general health indicator) TSH (general health indicator). Cortisol (to exclude Cushing syndrome) Prolactin (to exclude hyperprolactinemia). Dehydroepiandrosterone sulphate (to exclude Congenital Adrenal Hyperplasia (CAH)) 17-hydroxy progesterone. (to exclude CAH) Sex-hormone binding hormone (to calculate free androgen index) Testosterone (to diagnose PCOS-hyperandrogenemia) Androstenedione (alternate measure of hyperandrogenemia) Follicle stimulating hormone (FSH) (exclude other causes of anovulation/ amenorrhea Luteinizing hormone (LH) (as per FSH) Estradiol. (as per FSH) Beta human chorionic gonadotropin (exclude pregnancy) Fasting insulin (diagnose hyperinsulinemia) Fasting glucose (diagnose diabetes Hemoglobin A1C (glycosylated haemoglobin)
Where measures are taken?	Royal University Hospital (RUH) lab or any other SDH lab
Who takes the measures?	Lab technician at SDH lab.
Where does the analysis take place?	Will be analyzed by the Saskatoon Health Region Laboratory.
Appointment booking	Dr. Chizen will give a requisition form for “Diagnosis bloodwork”at the history/exam appointment
When measures are taken?	After initial diagnosis appointment .
Applicable appendices	None.

b) Routine PCOS Blood Measures: Analyzed at Saskatoon Health Region Laboratory

Specific measures	The blood for these measures will be collected at the RUH laboratory or other laboratory in the Saskatoon Health Region and will be analyzed by the Saskatoon Health Region Laboratory as part of routine medical care. These measures include: Beta human chorionic gonadotropin (diagnose pregnancy)

	Lipid screen	(fasting: cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides
	Sex-hormone binding hormone. Testosterone Androstenedione Follicle stimulating hormone (FSH) Luteinizing hormone (LH) Estradiol. Beta human chorionic gonadotropin (exclude pregnancy) Fasting insulin Fasting glucose Hemoglobin A1C	
Where measures are taken?	Royal University Hospital (RUH) lab or other SDH lab	
Who takes the measures?	Lab technician at SDH lab.	
Where does the analysis take place?	Will be analyzed by the Saskatoon Health Region Laboratory.	
Appointment booking	The kinesiology RAs will give the participants the requisitions for blood to be drawn during the program. Dr. Chizen will give the requisition at follow-up appointments.	
When measures are taken?	Fasting blood tests- early am Once at diagnosis appointment enrolment (w1). Once at the end of the sixteen-week program (bt w18- w20). Once at 6-month follow-up. Once at 12-month follow-up.	
Applicable appendices	None.	

c) Special Blood Measures That Will *Not* Be Measured at the Saskatoon Health Region Laboratory	
Specific measures	These blood for these measures will be collected by Dr Chizen in her office and blood samples will be processed in the RUH, SDH laboratory for later analysis by the investigators; the Saskatoon Health Region Laboratory will not be doing the analysis of these measures (results are not precise for research purposes). These fasting measures include: Estradiol. Androgen index (a ratio of testosterone to sex hormone binding globulin). FSH LH MG D-Lactate
Where measures are taken?	Dr Chizen's office.
Who takes the	Dr Chizen or Research assistant in Dr Chizen's office; Blood samples will be

measures?	transferred to the RUH laboratory on ice for processing and freezing. Frozen samples will be transferred from the RUH laboratory freezer to the investigators' freezer for storage until all samples can be analysed at completion of enrollment
Where does the analysis take place?	Technician at College of Pharmacy and Nutrition.
Appointment booking	The appointment will be made by Dr Chizen's research assistant or office assistant. The blood tests will be done fasting, at an early am time. If the volunteer has menstrual cyclicity, she will be asked to complete the tests between day 1-5 of her menstrual cycle. If no menstrual cyclicity exists, the appointment will be done at a random time of the menstrual cycle.
When measures are taken?	Once at enrolment (w1). Once at the end of the lead-in period (w4). Twice during the sixteen-week program (at w16 and w20). Once at 6-month follow-up. Once at 12-month follow-up.
Applicable appendices	None.
Transport of the stored blood	RAs with biohazard training will be responsible for picking up the stored samples twice a week.

d) Routine Metabolic Syndrome Blood Measures

Specific measures	The blood for these measures will be collected at the RUH laboratory and will be analyzed by the Saskatoon Health Region Laboratory as part of routine medical care (results are considered precise for research purposes). * All these measures should be taken when the participant is in a fasting state* These measures include: C-reactive protein. Hemoglobin A1C (HbA1C). LDL-cholesterol. HDL-cholesterol. Total Cholesterol. Triglycerides Insulin Glucose
Where measures are taken?	RUH lab.
Who takes the measures?	Lab technician at RUH lab.
Where does the analysis take place?	All these variables from plasma samples will be determined at the RUH laboratory.
Appointment booking	The participant will have the requisition for these lab measures given by Dr. Chizen at the appointment to diagnose PCOS; The kinesiology RAs will need

	to provide the requisitions for the measures that are to occur during the program. Dr. Chizen will send samples to the laboratory at the time of their GTT.
When measures are taken?	Fasting tests will be completed at the time of the 2 hour fasting GTT on 4 occasions: Once at enrolment (diagnosis of PCOS) (w1). Once during the lead-in period (w1-4). Once at the end of the sixteen-week program (w16 -w20). Once at 6-month follow-up. Once at 12-month follow-up.
Applicable appendices	None

e) Two-hour Blood Measures	
Specific measures	2- hr blood tests taken at 0, 30, 60, 90 and 120 minutes; Oral intake of 75 g glucose syrup will occur at time 0 min. Tests include: ▪ Insulin ▪ Glucose ▪ D-lactate ▪ Methylglyoaxal (MG).
Where measures are taken?	PCOS clinic (=WHIRL).
Who takes the measures?	Dr. Chizen (or research assistant) will draw the participant's blood in WHIRL. Blood samples will be transported to RUH lab for serum separation and immediate freezing.
Where does the analysis take place?	Location of final analysis to be determined.
Appointment booking	See sections 14.1, 14.3, 14.4 After the diagnosis of PCOS is confirmed by receipt of blood test results (see 18.6a), an appointment for the first 2hr fasting blood tests will be arranged with the participant by telephone call. Correspondence with the Kinesiology RA regarding confirmation of PCOS diagnosis will allow the Kin.RA to complete the enrollment procedure at a pre-arranged time. The kinesiology RAs and PCOS clinic RA will communicate to book appointments for the w18-20, 6month and 12 month fasting tests. (See section 14)
When measures are taken?	Once at enrolment (w1). Once at the end of the lead-in period (w4). Twice during the sixteen-week program (at w16 and w20). Once at 6-month follow-up. Once at 12-month follow-up.
Applicable appendices	None.
Transport of samples	Research assistant (Shani Serrao) will pick up frozen samples from RUH lab and transfer to WHIRL freezer (-70C) for temporary storage. Samples will be transferred from WHIRL freezer to

Nutrition freezer (-70C, room 331 thorvaldson).

(RAs with biohazard training will be responsible for picking up the stored samples twice a month.)

f) Saved Blood Measures
The analysis of these specific measures will occur if funds exist to conduct the analysis.

Specific measures	Fasting blood samples will be taken at the time of the 2hr GTT for the following tests: Adiponectin. Apolipoprotein B. AMH (anti-mullerian hormone). Homocysteine. Ghrelin. GLP-1. Leptin. Orexin. PPY. Small LDL.
Where measures are taken?	PCOS clinic(=WHIRL).
Who takes the measures?	Dr. Chizen (or research assistant) will draw the participant's blood in WHIRL. Blood samples will be transported to RUH lab for serum separation and immediate freezing.
Where does the analysis take place?	Location of final analysis to be determined.
Appointment booking	Fasting blood draws will be done at time 0 during performance of the 2hr GTT.
When measures are taken?	Once at enrolment (w1-4). Twice during the sixteen-week program (at w16 and w20). Once at 6-month follow-up. Once at 12-month follow-up.
Applicable appendices	None.
Transport of stored samples	Research assistant (Shani Serrao) will pick up frozen samples from RUH lab and transfer to WHIRL freezer (-70C) for temporary storage. Samples will be transferred from WHIRL freezer to Nutrition freezer (-70C, room 331 thorvaldson). (RAs with biohazard training will be responsible for picking up the stored samples twice a month.)

PROCEDURES FOR MONITORING PARTICIPANT COMPLIANCE

There are two study diaries (logs).

19.1 Study Log 1 (Appendix N) is for the lead-in phase (when participant has only received the TLC guidelines).

19.2 Study Log 2 (Appendix O) is for the study phase (when participant is participating in the diet and exercise program). Participant's compliance with following the TLC guidelines will be recorded in the study log 2. The participant will record whether or not they consumed their servings each day. If partial servings were consumed, this will be recorded as estimated percentage.

19.3 Compliance to the exercise-training program will be monitored via a daily exercise in Log 2. The kinesiology RAs will keep a calendar to track the attendance record of participants. Participants will also complete compliance to supervised exercise in the Aerobic Tracking Logs (Appendix L).

FOLLOW-UP

20.1 We will meet with participants at 6 months and 12 months to determine if they continue to follow the "standard of care" recommendations (i.e. TLC diet and exercise) and make observations of metabolic syndrome and PCOS symptoms. Participants who were randomized to the pulse-based diet will be asked if they continue to follow a pulse-based diet. Participants randomized to receive the low cholesterol (non-pulse based) diet will be asked if they continue to follow the low cholesterol diet.
20.2With our sample size, we anticipate that we will be able to statistically determine differences between individuals who continued to follow the pulse-base diet vs individual who did not continue a pulse-based diet.

20.3 Kinesiology RAs will set up the 6-month and 12-month follow-up appointments for the participants. The participants will attend the PCOS clinic, where the participant will:

- Undergo a medical evaluation by Dr. Chizen
- Receive a requisition for routine bloodwork from Dr. Chizen
- Receive a transvaginal ultrasound
- Undergo the fasting 2-hour blood draws
- Receive a package with the food record, leisure-time questionnaire (include postage-paid, return addressed envelope)
- Receive instructions for accessing the online questionnaires

STATISTICAL PROCEDURES

21.1 All raw data will be compiled (on an Excel Spreadsheet) and reviewed for accuracy. Descriptive statistics will be performed for all outcome measurements for the presentation of group data (e.g. means, SD, etc) and to identify outliers as well as determination of normality of the data (for application of either parametric or non-parametric inferential statistics).

21.2 Inferential statistics will be carried out to determine if differences exist between the two study groups. Baselines measures (dependent variables) will be compared to ensure similarity between the two groups (i.e., unpaired t-test).

21.3 Over the course of the study period (baseline, intervention, follow-up), the treatment effect on the dependent variables will be assessed by a group by time ANOVA, with repeated measures on the time factor (SPSS – Statistical Package).

21.4 Dietary intake data will be analysed for energy and nutrient content using the dietary analysis program Food Processor (version 7, ESHA Research, Oregon). This program enables the use of both the USDA Handbook 8 (USDA, 1999) and the Canadian Nutrient File (CNF 1997 version). The database includes more than 20,000 food items, including specialty, ethnic, combination and fast foods. Other food items also can be entered from recipes. Analyses can be performed for greater that 130 nutrients and nutrient factors. Intake data obtained will be used not only to monitor pulse consumption but also to evaluate the adequacy of the diet in comparison to the Dietary Reference Intakes (DRIs).

21.5 Questionnaire data (Quality of Life, Pulse Consumption, Physical Activity) will be descriptively presented. Appropriate analyses will be carried out to monitor changes in the questionnaire responses.

21.6 Data will be analysed on an intent-to-treat basis; that is, an attempt will be made to re-measure participants that did not adhere to the exercise or supplementation. An additional valid completers analysis will be done including only those Participants completing the entire study and who were compliant with their assignment.

KNOWN AND POTENTIAL RISKS AND DISCOMFORTS TO PARTICIPANTS

22.1 Possible side effects from the ultrasound examination include minor discomfort upon insertion of the transvaginal probe and a transient feeling of pressure in vagina or on belly.
22.2 Possible side effects from blood drawing include fainting, inflammation of the vein, pain, bruising or bleeding at the site of puncture.
22.3 Exercise may result in muscle pulls, strains, or soreness. Participants will be given a proper warm-up prior to and qualified exercise trainers will supervise training sessions to minimize any risk. Adequate rest will be given between training and testing sessions to ensure that the participants' muscles are recovered by the next training session. Training will initially be light and will gradually increase over the first four weeks to allow muscles to get used to the training and minimize muscle soreness. See Appendix R: Fitness Testing Data Form.
22.4 There is a small amount of radiation exposure from the dual energy X-ray scans. This is equal to one tenth of the amount of radiation one would receive from taking a trans-Atlantic flight from North American to Europe, or less than 0.5% from what one would receive from a routine full-mouth dental X-ray.
22.5 The pulse-based diet is a high fiber diet; therefore, if one is not used to high fiber foods they may initially experience some gastrointestinal discomfort and flatulence.

22.6 By not taking oral contraceptives, there is a risk of becoming pregnant if no other form of birth control is used

22.7 The questionnaires ask personal questions that one may feel uncomfortable answering. Participants have the option of not answering any questions that cause them discomfort or distress.

STATEMENT ON COMPLIANCE WITH PROTOCOL AND GOOD CLINICAL PRACTICE

This trial will be conducted in compliance with the protocol and Good Clinical Practice (GCP).

BLINDING

24.1 This is a single blind study. Participants will be aware of the diet and exercise intervention they are receiving. Research assistants involved with the training will also not be blind. However, the investigators who will be conducting the analyses will remain blind to the participants' group assignments.

24.2 The allocation list will be concealed from the kinesiology RAs, as they will be responsible for enrolling participants. The kinesiology RAs will keep the master list of which groups the participants are in.

RANDOMIZATION

Randomization will be done with a computer-generated allocation schedule with a block size of 4, making sure that there are equal numbers of subjects on each type of medication across the two groups.

ETHICAL APPROVAL

Ethical approval was granted by the University of Saskatchewan Ethics Review Board for Biomedical Research in Human Subjects (BIO #10-98) on October 21, 2010.

OPERATIONAL APPROVAL

Operational approval was granted by the Saskatoon Health Region on December 14, 2010.

CLINICAL TRIALS REGISTRATION

This trial has been registered with clinicaltrials.gov. It is necessary to update the status of the trial (i.e., when recruitment begins and ends, when study closes, etc.). Lindsay Tumback has the password to access the clinical trials record.

QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

29.1 A RA will be in charge of data cleaning - checking for mistakes (based on predetermined acceptable data ranges) and omissions prior to making entries into the database. The primary investigator will be notified if there are invalid or missing values.

29.2 When data corrections and modifications are made, the following will be recorded: the change made, the person initiating the change, and the explanation for the change. The database will be backed up daily by the RA. Participant data will be chosen at random and compared to that entered into the database a second time for quality control purposes.

MEDICATIONS / TREATMENTS PERMITTED BEFORE OR DURING THE TRIAL

30.1 Participants will be excluded from participation in the study if using the following medications during the 3 months prior to at the time of the study:

- 30.1.1 Any hormonal contraception or fertility medications
- 30.1.2 Use of anticonvulsant medications or psychotropic medications known to increase prolactin, increase insulin resistance and induce polycystic ovaries
- 30.1.3 Use of estrogen or continuous progestin or androgen hormones
- 30.1.4 All prescription medications used by the participant will be reviewed by Dr Chizen to determine if there are reasons to restrict use during the study or to exclude participation from the study

31.2 All other medications are permitted. The study logs will prompt the participant to disclose any prescribed, herbal, or over the counter medication changes on a weekly basis.

31. Visit Protocol in Chronological Order

31.1 Phone conversation about study between potential participant and Kin-RA
31.2 Phone conversation from Kin-RA with Dr Chizen/WHIRL to arrange PCOS diagnosis appt
31.3 Phone call to potential participant to communicate time of PCOS diagnosis appointment and to arrange time to sign consent
31.4 Participant meets with Kin/NutRA to discuss study and sign consent
31.5 Participant appointment with Dr Chizen/WHIRL to diagnose PCOS: history, exam, ultrasound and receive fasting blood test requisitions (45+ min)
31.6 Chizen/WHIRL receives all blood tests results and records in database backed up on SDH/WHIRL server; (will send later as excel file)
31.7 Phone call from Dr Chizen/WHIRL to participant to arrangeappointment for fasting 2 hr blood tests and ultrasound
31.8 Phone call/email to Kin RA from Chizen/WHIRL to arrange enrolment appointment—

31.8.1 If enrolment appointment is not arranged during 31.5/31.6, Kin RA will contact participant

31.9 Participant appointment for fasting 2 hour blood tests (GTT) and ultrasound exam with Chizen/WHIRL (booked for minimum 135-145 min)

31.10 Kin RA will arrange time for enrolment measures/ tests (anthropometric, DEXA and will give instructions for accelerometer,

31.11 Kin RA will arrange time for TLC education and provide diet questionaire

31.12 Kin RA will arrange times for exercise 3x/wk

APPENDIX A: Participant Information Sheet and Consent Form

RESEARCH PARTICIPANT INFORMATION SHEET AND CONSENT FORM

A Lifestyle Intervention for Women with Polycystic Ovary Syndrome: The Role of a Pulse-Based Diet and Aerobic Exercise on Infertility Measures and Metabolic Syndrome Risk

Funded by: Agriculture and Agri-Food Canada (Growing Canadian Agri-Innovation Program) and Pulse Growers of Saskatchewan

RESEARCH TEAM

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The twenty-four hour contact phone number for this study is 230-3849 (Dr. Chilibeck)

INTRODUCTION

You are invited to take part in this research study because you have symptoms of Polycystic Ovary Syndrome (PCOS). Your participation is completely voluntary and it is up to you whether or not you wish to take part in it. Before you give your consent to participate in the study, please read the following information sheet and ask as many questions as necessary to be sure that you understand what your participation will involve. Please feel free to discuss this with your family, friends, or family doctor before you decide.

If you decide to participate, you are still free to withdraw at any time without giving any reasons for your decision. If you do not wish to participate, it will not affect your current medical care.

WHO IS CONDUCTING THE STUDY?

The study is being conducted at three sites: the Women's Health Imaging Research Laboratory (WHIRL) in the Department of Obstetrics, Gynecology and Reproductive Sciences at the Royal University Hospital, the College of Pharmacy and Nutrition, and the College of Kinesiology at the University of Saskatchewan. The Principal Investigators will not receive any payment to conduct this study. Hence there is no monetary gain to the investigator if you should decide to become a participant.

WHY IS THIS STUDY BEING DONE?

Some metabolic complications are higher in women with PCOS than the general population. They include increased cholesterol levels, type 2 diabetes, and metabolic syndrome. Metabolic syndrome occurs when a person has insulin resistance, high blood pressure, abnormal blood fat levels, and abdominal obesity. These factors increase the risk for cardiovascular (heart) disease.

The cause of PCOS is not fully understood; however, high blood insulin (a hormone) is believed to play a role. Higher amounts of insulin can lead to increased fat storage (and obesity) and stimulate male hormone production. Too much male hormone can increase male pattern hair growth, interfere with ovulation and cause unpredictable menstrual periods. Suggested treatment of PCOS includes nutrition and exercise lifestyle changes to reduce PCOS symptoms and metabolic syndrome risk.

Our study is designed to compare two different lifestyle programs for women with PCOS. Program 1 includes a heart-healthy diet (the Therapeutic Lifestyle Changes diet) in combination with aerobic exercise. Program 2 combines the heart-healthy diet and a pulse-based diet (e.g., lentils, chick-peas, peas, and beans) and aerobic exercise.

We would like to observe how each diet and exercise program affects your body shape, hormone levels and the appearance of your ovaries. We want to know if your diet and exercise patterns change during and after the study. We would also like to understand what you learned from the study and will ask for your opinions about participating in the study after the study ends.

WHO CAN PARTICIPATE IN THE STUDY?

If you have been diagnosed with PCOS and are between the ages of 18 and 35 years, you are eligible to participate in this study. For a diagnosis of PCOS, two of three criteria must be present: 1) irregular or absent menstrual cycles, 2) high blood level of male hormone or male pattern hair growth, and 3) polycystic ovaries (enlarged ovaries with multiple small follicles (egg sacs) in the ovary) confirmed by transvaginal ultrasound examination.

If you are taking birth control medication, or male or female (estrogen-containing) hormones or have allergies to a pulse-based diet, you are excluded from the study. If you wish to participate in the study, you should use contraception to avoid becoming pregnant during the diet and exercise program. You must not use any hormonal contraception or sex-hormone supplements or fertility medications for three months prior to the study or during the study. Birth control, sex hormones and fertility medications are not permitted because ovarian follicle growth is altered by these medications. Women who take some anti-seizure medications or some medications used to treat mood disorders may also be excluded because these medications can change the number of ovarian follicles. If the ultrasound examination shows a follicle that may ovulate, you will be advised to use a barrier contraceptive method to prevent pregnancy during the diet and exercise program (for example, condoms or diaphrams and spermicidal agents). We ask that you tell us about any medications (including herbal formulations) you take as some medications can affect follicle growth and egg release.

WHAT DOES THE STUDY INVOLVE?

1. Medical Evaluation
To determine if you are eligible for this study, you will have a medical evaluation visit with co-investigator Dr. Donna Chizen in the Department of Obstetrics, Gynecology, and Reproductive Sciences. This visit will take approximately 30 to 45 minutes. You will be asked about your medical and menstrual history. Your body type and hair growth pattern will be assessed according to a standard medical scoring system. Your vital signs (pulse, respirations, temperature, blood pressure), height, and weight will be measured. A transvaginal ultrasound (a procedure where sound waves are sent out by an ultrasound probe that has been inserted in the vagina) will be completed to help with the diagnosis of polycystic ovaries. A diary card will be given to you at this visit, to monitor menstrual cycles and any health problems. Any health problems will be reviewed, assessed, and documented by the investigators throughout the study.

You will be asked to have some blood tests at a Saskatoon Health Region Laboratory after this consultation. Dr Chizen will arrange time to review the test results with you and you will have the opportunity to ask questions. If the tests confirm that you have PCOS, we will ask you to fast for 12 hours to complete other blood tests done by Dr. Chizen. These tests can identify health risk factors typically seen in women with PCOS. One of the tests, the Glucose Tolerance Test, is used to diagnose insulin resistance and diabetes. Approximately 2 tablespoons of blood will then be collected. Next, you will be asked to drink glucose syrup, followed by collection of 7 ml (one teaspoon) of blood every 30 minutes for 2 hours. We will also test to determine if you are pregnant, if you have abnormal blood fats, or if you have extra male hormone from your adrenal glands or ovaries. With your permission, if any abnormalities are discovered, we will notify your current primary care physician or make a referral to an appropriate physician for follow-up medical care. A follow-up medical evaluation may be scheduled.

2. Initial Visit with Study Coordinator
If Dr. Chizen has diagnosed PCOS, she will direct you to the study coordinator. You will have your height, weight, waist circumference, heart rate and blood pressure recorded. Next you will have your body composition (whole-body lean tissue and fat mass) assessed. You will be given an accelerometer (a small device that measures your physical activity) to wear for one week (these two measures will be described in more detail below). You will complete a few questionnaires.

3. Randomization to Lifestyle Program
You will be randomly assigned (by chance as determined by a computer) to one of the two programs:

If you are assigned to program 1, you will be asked to follow the Therapeutic Lifestyle Changes (TLC) guidelines. You will be given a $40 grocery card every week throughout the intervention period for about 16 weeks so that you can purchase the foods necessary to follow the TLC diet. You will be asked to attend a 90-minute diet education session, where a Registered Dietitian will explain the TLC guidelines. This education session will occur at the Williams Building (a map will be provided). The first two weeks of the program are referred to as the “lead-in period” (the period of time where all participants follow to the TLC guidelines). Following the “lead-in period” you will be asked to continue the TLC guidelines and consume the TLC diet for 16 weeks. The TLC diet will be nutritionally balanced and will contain lean meats as the protein source. Nutritional counseling will be provided to you every two weeks throughout the intervention period of 16 weeks.

If you are assigned to program 2, you will be asked follow the Therapeutic Lifestyle Changes (TLC) guidelines and you will receive a pulse-based diet. You will be asked to attend a 90-minute education session, where a Registered Dietitian will explain the TLC diet guidelines. This education session will occur at the Williams Building (a map will be provided). The first two weeks of the TLC diet are referred to as the “lead-in period” (the time where all participants follow to the TLC guidelines). Following the “lead-in period”, you will be asked to follow the TLC diet for 16 weeks in addition to consuming a pulse-based diet. The pulse based-diet will include meals prepared with dry peas, lentils, chickpeas, and/or beans. Two meals will be supplied daily for 16 weeks to those participants on the pulse-based diet program. Meals will be nutritionally balanced and will contain pulses as the protein source. The meals will contain approximately 90g dried peas, 225g chickpeas or beans, or 150g lentils.

4. Exercise Program

All participants will follow an exercise program for 16 weeks. The program will involve 5 sessions of aerobic exercise per week: 3 sessions of supervised exercise training and 2 sessions of unsupervised at-home exercise per week. Both the supervised and unsupervised exercise will be at the same intensity: at least 60-75% of your age-predicted maximal heart rate (220-Age). The supervised exercise training will occur in the College of Kinesiology at the Williams Building (treadmill walking and exercising on a rowing machine and exercise bike). The unsupervised exercise will involve walking (or other types of aerobic activity if the participant has access to aerobic exercise equipment at home). Each exercise session will be 45 minutes long.

5. Measures

a) Ultrasound Examinations

The study involves 5 ultrasound examinations that will take approximately 10 minutes each. Transvaginal ultrasonography will be used. The procedure involves inserting an ultrasound probe into the vagina to take pictures of your ovaries and uterus. The ultrasound exams will be performed by co-investigators Dr. Chizen or Dr. Pierson in the Ultrasound Imaging Suite in the Department of Obstetrics and Gynecology at Royal University Hospital (Room 4511). The examinations will occur at the PCOS diagnosis appointment, at the start of the study, at the end of the diet program, and at 6 and 12-months follow-up (after the diet program ends).

b) Blood Tests

You will be asked to have some blood tests at a Saskatoon Health Region Laboratory or in Dr Chizen’s office. Tests will be done to diagnose PCOS, prior to the start of the diet program, at 9 weeks into the diet program, the end of the diet program, and at 6 and 12 months after the diet program ends. These tests can identify health risk factors typically seen in women with PCOS. We will determine if you are pregnant, have diabetes or insulin resistance, if you have abnormal blood fats, or if you have extra male hormone from your adrenal glands or ovaries. Dr Chizen will share the test results with you. With your permission, Dr Chizen will notify your current primary care physician if any abnormalities are discovered, or she will refer you to an appropriate physician for follow-up medical care.

c) Body Composition Assessments

We will measure your body composition (lean tissue and fat mass) with dual energy X-ray absorptiometry four times. This examination involves you lying still on a padded table for about 10 minutes while a low energy X-ray beam scans your body. After this scan, we will assess your blood pressure and measure your waist girth. This assessment will take about 30 minutes. The body composition assessments will occur at the start of the study, at the end of the diet program, and at 6 and 12-months follow-up (after the diet program ends).

d) Physical Activity Assessments

You will be given a questionnaire called the “Physical Activity Readiness Questionnaire” to see if you have any health risks that would make it dangerous to exercise. If you indicate any health risks, with your permission, we will contact your family physician to give you clearance to participate in the exercise program. Five times during the study you will be asked to complete a 1-2 minute questionnaire called the “Leisure-Time Exercise Questionnaire.” The questionnaire will be given the start of the study, one time during the diet program, once at the end of the diet program, and at 6 and 12-months follow-up (after the diet program ends).

e) Diet Assessments
On 9 occasions during the study you will be asked to record the food that you have eaten for one to four days. Assessments will occur at the start of the study, twice during the TLC diet, 4 times during the study diet, and at 6 and 12-months after the study diet ends.

f) Quality of Life and Motivation Assessments
Quality of life and motivational questionnaires will be administered at the start of the study, at the end of the diet and exercise program, and at 6 and 12 months follow-up. The questionnaires will be administered via a secured and confidential website run through the University of Saskatchewan. You may complete the questionnaires on your home computer, or you may have access to a study computer in a private area. It will take approximately forty minutes to complete the questionnaires.

g) Pulse-Consumption Questionnaire
You will be asked to complete the Pulse Consumption Questionnaire to determine how often you eat pulse foods. The dietitian will describe the different types of pulse foods. The questionnaire will be given at the start of the study, at the end of the diet and exercise program, and at 6 and 12 months follow-up. The questionnaire takes approximately 20 minutes to complete.

h) Activity Measurement
Your activity will be measured for one week on three occasions using the Actical accelerometer: Once when you enroll in the study, and 12 months after the diet intervention is completed. The Actical is a small activity monitor (it is the size and dimensions of a small pager), that is worn on a belt around your waist. We will explain how to use the monitor and will also give you written instructions. You will be asked to wear the monitor at all times while awake, except when bathing, swimming or sleeping. You will be asked to record the time the monitor was attached and removed to calculate your activity and sleeping times.

SUMMARY OF TIME COMMITMENT

A summary of the total approximate time commitment throughout the study is as follows:
- Initial medical appointment with Dr. Chizen (30-45 minutes)
- Exercise training 45 minutes per session, 5 sessions per week for 16 weeks
- 500 minutes of testing including blood tests and ultrasound examination, DEXA scans, body measurements, and exercise testing done on 4 occasions
- Attendance at TLC diet education session: 90 minutes
- Total time for diet assessments= 20 minutes per food record on 9 occasions
- Total time for physical activity assessments= 2 minutes per assessment on 8 occasions
- Motivation, quality of life, and pulse consumption questionnaires= 60 minutes on 4 occasions

Total time commitment is approximately 81 hours over 16 months (including follow-up).

Week # or phase	Diagnosis	Pre-TLC	TLC week 1	TLC week 2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	6-months after end of w20	12-months after end of w20
Ultrasounds	X	X											X							X	X	X
DEXA		X																		X	X	X
BP and WC		X																		X	X	X
Weight and height		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Accelerometers		X																				X
Par-Q		X																				
Leisure Time Questionnaire		X						X				X				X				X	X	X
1-day Food Record			X	X				X				X				X				X		
4-day Food Record		X																			X	X
Pulse Consumption Questionnaire		X																		X	X	X
Other Questionnaires	X																			X	X	X
Routine PCOS Blood Measures	X			X									X							X	X	X
Routine Metabolic Syndrome Blood Measures	X			X									X							X	X	X
Two-hour Blood Measures		X																		X	X	X
Saved Blood Measures	X	X		X									X							X	X	X
Diet Counselling (TLC group only)						X		X		X		X		X		X		X		X		

DEXA = dual energy x-ray absorptiometry, BP = blood pressure; WC = waist circumference; PAR-q = physical activity readiness questionnaire

WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

The benefits to you for participation in this research study include a diet and exercise program. You will be given advice regarding your personal risk factors for long-term health problems. You will be informed about ways to improve your health and to control undesired hair growth, weight, menstrual cycle regularity and fertility. It is hoped the information gained from this study can be used in the future to benefit other women with PCOS.

You do not have to participate in this study to reduce your risk of metabolic syndrome. You can exercise on your own or arrange for an appropriate program with an exercise trainer to reduce your risk of metabolic syndrome. You can also alter your diet by reducing saturated fats or take medication to lower your blood cholesterol level and blood pressure.

WHAT ARE THE COSTS OF PARTICIPATING IN THIS STUDY?

You will not be charged for any study-related procedures. You will not be paid for participating in this study. Parking costs for the medical assessments, and lab tests will be reimbursed with the submission of the original receipts.

ARE THERE POSSIBLE RISKS OR DISCOMFORTS?

- Possible side effects from the ultrasound examination include minor discomfort upon insertion of the transvaginal probe and a transient feeling of pressure on your vagina or inside your belly.
- Possible side effects from blood drawing include fainting, infection, bruising or bleeding of a vein at the site of skin puncture. You may be given a heating pad to make it easier to see blood vessels.
- Exercise may result in muscle pulls, strains, or soreness. You will be given a proper warm-up prior to and qualified exercise trainers will supervise training sessions to minimize any risk. Adequate rest will be given between training and testing sessions to ensure that your muscles are recovered by the next training session. Training will initially be light and will gradually increase over the first four weeks to allow your muscles to get used to the training and minimize muscle soreness.
- There is a small amount of radiation exposure from the DEXA scan (dual energy X-ray absorptiometry scans). This is much weaker than a typical chest or bone X-ray and is equal to one tenth of the amount of radiation you would receive from taking a trans-Atlantic flight from North American to Europe, or less than 0.5% from what you would receive from a routine full-mouth dental X-ray.
- The pulse-based diet is a high fiber diet; therefore, if you are not used to high fiber foods you may initially experience some gastrointestinal discomfort and flatulence.
- By not taking oral contraceptives, there is a risk of becoming pregnant if no other form of birth control is used. We ask that you use contraception regularly to avoid the opportunity to become pregnant while you are in the study.
- The questionnaires ask personal questions that you may feel uncomfortable answering. You have the option of not answering any questions that cause you discomfort or distress.

RESEARCH RELATED INJURY

In the case of a medical emergency related to the study, you should seek immediate care and, as soon as possible, notify the principal investigator. Necessary medical treatment will be made available at no cost to you. By signing this document, you do not waive any of your legal rights.

HOW WILL MY PARTICIPATION BE KEPT CONFIDENTIAL?

You will be identified only by your assigned research participant study number. The results of this study may also be used for medical and scientific publications; the data will be grouped with that of the other participants and your identity will not be disclosed.

With your permission, we will forward a letter to your family doctor's office to inform him/her of your participation in this study and your PCOS condition, unless you specifically request otherwise. We will notify your family doctor if we discover any information during the course of the study that may be useful for your medical care.

WHAT IF I DECIDE TO WITHDRAW?

You may decide not to participate, or may withdraw at any time. Your refusal to participate in, or your withdrawal from, the study will not affect your future medical care. If you wish to withdraw from the study, please notify any member of the Research Team as soon as possible.

Your participation in this study may be ended at any time without your consent. Reasons may include, but are not limited to, your failure to follow study instructions, adverse events not related to the study, or study cancellation due to administrative reasons.

If you are a student, staff, or faculty member at the University of Saskatchewan and you decide not to participate or withdraw your consent later, this will not affect your academic standing, employment, promotion, or the services you could otherwise expect to receive at the University.

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY?

If you have any questions regarding your participation in this study, please feel free to contact Dr. Phil Chilibeck by phone at 966-1072, cell: 230-3849; or by email at phil.chilibeck@usask.ca.

If you have any questions about your rights as a research subject or concerns about the study, you may contact the Chair of the Biomedical Research Ethics Board, care of the Ethics Office, University of Saskatchewan at (306) 966-4053 (collect calls accepted). The Research Ethics Board is a group of individuals (scientists, physicians, ethicists, lawyers and members of the community) that provide an independent review of human research studies. This study has been reviewed and approved on ethical grounds by the University of Saskatchewan Biomedical Research Ethics Board.

Consent Form

I have read and understood the attached Patient Information Sheet. I freely and voluntarily agree to take part in the study entitled “A Lifestyle Intervention for Women with Polycystic Ovary Syndrome: The Role of a Pulse-Based Diet and Aerobic Exercise on Infertility Measures and Metabolic Syndrome Risk”.

I have received an explanation of the purpose and duration of the trial, and I am aware of the potential benefits and side effect associated with this study.

I was given sufficient time and opportunity to ask questions and to reflect on my understanding of participation in the study. My questions have been answered to my satisfaction.

I agree to cooperate fully with the study personnel and will tell him/her of any medicine, drug, or alternative therapy (herbal remedy) of whatever nature I have taken in the recent past, or am taking now, whether prescribed or not.

I have been given a copy of the Patient Information materials and will be given a copy of this signed and dated Consent Form. I am free to withdraw from the study at any time, for any reason, and understand that this will not affect my future medical treatment, my academic standing or employment.

Study personnel or other regulatory authorities may wish to review my medical records to verify the information collected. I have been assured that my name, address, and telephone number will be kept confidential to the extent permitted by applicable laws and/or regulations. By signing and dating this document, I give permission for such review and data collection, and grant direct access to my medical records. I am aware that none of my legal rights are being waved.

Please check and initial the appropriate box to indicate your decision:

__ ____Yes, I agree that you may inform my family doctor of my participation in this study

__ ____No, I do not want you to inform my family doctor of my participation in this study

Signature of research participant: ____________________________Date:___________________

Printed name of above: _________________________________

I confirm that I have explained the purpose and procedures of this study, as well as any potential risks and benefits, to the subject whose name and signature appears above.

Signature of person conducting the consent process: _______________________

Date: _________________

Printed Name of Above: ___________________________ Study Role: _________________

Appendix B: Researcher's Summary Form
* Note: the Appendices mentioned in this Researcher's Summary are not included.

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board

RESEARCHER'S SUMMARY FORM

Bio-REB File Number: ____10-98_________

PROJECT TITLE: **A Lifestyle Intervention for Women with Polycystic Ovary Syndrome: The Role of a Pulse-Based Diet and Aerobic Exercise on Infertility Measures and Metabolic Syndrome Risk**

PRINCIPAL INVESTIGATOR: Gordon Zello, Ph.D., Professor, College of Pharmacy and Nutrition

DEPARTMENT: College of Pharmacy and Nutrition

SUB-INVESTIGATOR(S):
Philip D. Chilibeck, Ph.D., Associate Professor, College of Kinesiology
Roger Pierson, MS PhD FEAS FCAHS, Professor, College of Medicine
Donna R. Chizen, MD FRCSC, Associate Professor, College of Medicine
Karen Lawson, Ph.D., Associate Professor, Department of Psychology, College of Arts and Science

STUDENT RESEARCHER(S): Lindsay Tumback, MSc, RD, PhD Student, College of Pharmacy and Nutrition

DEPARTMENT: College of Pharmacy and Nutrition

RESEARCH WILL BE CONDUCTED AT: College of Pharmacy and Nutrition, College of Medicine and College of Kinesiology

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1. **<u>Hypothesis</u> (State briefly the nature and purpose of the research proposal, and the proposition the research is seeking to uphold. What potentially useful knowledge or clarification about therapeutic options will be advanced to justify the participation of human subjects in this research project?)**

The purpose of this study is to evaluate a lifestyle intervention for women with polycystic ovary syndrome (PCOS). There are five main objectives of the proposed study:

1. To characterize the lifestyles (i.e. diet including pulse consumption and exercise), quality of life, other health indicators for risk of disease (e.g. blood parameters, anthropometry, body composition) and infertility in a large sample (n>150) of women with PCOS (from baseline measures)
2. To determine the short-term therapeutic effects of a pulse-based diet on the multiple disease measures of PCOS and metabolic syndrome after 16 weeks.
3. To determine the long-term therapeutic effects of a pulse-based diet on the multiple disease measures of PCOS and metabolic syndrome through follow-up measurements of the participants (at 6 months and 12 months post-intervention)
4. To measure the effect of a pulse-based diet intervention on participants' consumption of pulse foods after completion of the intervention diet.
5. To measure the effect of a pulse-based diet on quality of life scores of women with PCOS during the intervention and at 6 months and 12 months follow-up.

Our hypotheses are:

1. Introduction of a pulse-based diet for 16 weeks will reduce hyperinsulinemia and improve other markers of the metabolic syndrome (i.e. reduce glucose, triglycerides, blood pressure, abdominal fat, and increase HDL); and therefore reduce the disease measures, symptoms of PCOS (i.e. increase number of ovarian follicles, increase levels of estradiol and progesterone and decrease androgens).
2. Women with PCOS who are given a 16 week pulse-based diet will continue practicing the consumption of pulses for 6 and 12 months after the intervention and this will continue to improve their resting insulin levels, markers of metabolic syndrome, and symptoms of PCOS.
3. The pulse-based diet will increase the quality of life of women with PCOS.

2. Academic Validity (Provide evidence that the scientific reasoning and design of the project are sufficiently sound to meet the objectives of this project. Provide your own comments and if possible those resulting from peer review. Indicate if any other committee or agency has assessed the project's scientific validity):

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age, affecting approximately 105 million women worldwide (Azziz, 2005) and an estimated 1.4 million women in Canada (Lujan et al, 2008). Women with the syndrome present with several clinical signs, which include menstrual and ovulatory dysfunction, hyperandrogenemia (excess male sex hormones), hirsutism (male-pattern hair growth in women e.g. beard or chest hair), polycystic ovaries, insulin resistance, and hyperinsulinism (Azziz et al., 2009). A diagnosis of PCOS is accompanied with an increased risk of severe and devastating consequences such as infertility, dysfunctional bleeding, endometrial carcinoma, obesity, type 2 diabetes mellitus (DM), dyslipidemia (alterations in blood lipids), hypertension, and possibly cardiovascular disease (CVD) (Azziz, et al., 2009; Ehrmann, 2005; Liepa, 2008; Lujan et al, 2008).

The pathogenesis of PCOS is not fully understood; however, insulin resistance and associated hyperinsulinemia are believed to be the key contributing factors (Douglas, 2006; Moran & Norman, 2004). Insulin resistance and the subsequent production of excessive amounts of insulin are directly associated with the increased ovarian production of androgens and the features of PCOS (Blank, McCartney, & Marshall, 2006). Between 50% and 70% of women with PCOS exhibit insulin resistance.

Metabolic complications of insulin resistance are higher among women with PCOS than the general population and include the development of dyslipidemia (i.e. increased cholesterol levels), type 2 diabetes mellitus, and metabolic syndrome (Azziz et al., 2009; Ehrmann, 2005). Metabolic syndrome is a clinical diagnosis for an individual who exhibits the risk factors of insulin resistance, hypertension, abnormal serum lipid levels (i.e. high triglycerides and low high-density lipoproteins) and abdominal obesity (Liepa, 2008). In PCOS women with metabolic syndrome testosterone and serum androgen are higher than in non-metabolic syndrome PCOS women, reflecting more severe insulin resistance and PCOS disease features in those with metabolic syndrome. In addition, the presence of metabolic syndrome places women with PCOS at higher risk of cardiovascular disease (Apridonidze, Essah, Iuorno, & Nestler, 2005).

Therefore, lifestyle modifications, including diet and exercise, are essential for effective management of PCOS symptoms. Hyperinsulinemia and adiposity exacerbate the underlying metabolic and reproductive abnormalities (Lau, 2007) and many of the medical therapies for PCOS are less effective in women who are obese (Palomba, 2008). Thus, short-term dietary management of PCOS involves control of body weight and addressing PCOS symptoms and infertility. Long-term management of PCOS must also address the increased risk of the aforementioned metabolic complications and the chronic diseases that afflict this vulnerable group of women (Marsh, 2005).

The proposed study will examine a pulse-based diet as a nutrition intervention for the PCOS population. The rationale for this intervention is supported by the association of pulse-based diets with improved glycemic control (Sievenpiper et al. 2009). The use of a pulse-base diet is further supported by data originating from our research group which has shown positive effects of including pulses in the diet of individuals at risk for metabolic syndrome.

If applicable, please indicate whether Health Canada (TPD) approval has been obtained:

Yes _____ No _____ Pending _____ N/A _X____

3. **<u>Funding</u> (indicate the source of funds supporting the research. If externally funded, state whether the grant or contract is still in application, or has already been awarded):**

The project has been approved for funding under the Pulse Science Cluster (PSC) initiative. The project is financially supported by Agriculture and Agri-Food Canada (AAFC).

4. <u>Disclosure of Potential Conflicts of Interest</u> (indicate any motivation or incentives for conducting this study that arise external to the objectives of the study, e.g., will the investigator or institution be paid to conduct this research project? <u>Note</u>: The consent form should also include an introductory disclosure of potential conflict of interest statement, where applicable, indicating that this is a medical research study for which the study doctor, the institution, or both are being paid):

None.

5. <u>Subjects</u>

a) Target Population (e.g., age, gender, medical condition, target enrollment, significant inclusion/exclusion criteria):

We have established a target recruitment of 166 women (n= 83 per group). Inclusion criteria include a diagnosis of PCOS and be aged 18-38 years. An age limit of 38 years was established because the fertility measures of this study are affected by age (Canadian Fertility and Andrology Society, 2004). Women who are taking birth control or fertility medications with medical conditions that limit exercise or which limit consumption of a pulse-based diet (allergies or intolerances) are excluded from the study.

b) Proposed Strategies for Recruitment (e.g., use of advertisements, brochures, physician patient records):

Two of the researchers on the team, Drs. Pierson and Chizen, frequently conduct research with women with PCOS. Women will be recruited through posters throughout the University of Saskatchewan, physician offices, health care establishments, and advertisements in local newspapers (Appendix A).

6. **<u>Procedures</u> (clearly identify treatment allocation design, and describe the medical and other procedures to be followed in obtaining research data, including questionnaires):**

Recruitment

Women who respond to the advertisement will be scheduled to attend a PCOS clinic time and asked to undergo a medical evaluation to determine the presence of PCOS. All women will receive the results of the diagnostic tests and will be called in for a follow-up appointment. Only those women who have been diagnosed with PCOS will be asked to provide consent to participate in the study and to have their assessment data used in the analysis.

Allocation Design

The study is a single-blind parallel stratified-randomized design. Randomization will be stratified based on medication use (i.e. medications for PCOS symptoms, glucose control, lipid-lowering, or hypertension and those who are not taking medications).

Participants will be randomly assigned to one of two lifestyle programs:
1) Participants in Program 1 will receive the placebo diet (n= 83)
2) Participants in Program 2 will receive the pulse-based diet (n= 83)

Standard of Care
All participants will also receive supervised aerobic exercise training to provide recommended standard of care and to reduce variability in this lifestyle factor between participants. To control for diet variability, all participants will be instructed to follow the dietary recommendations of the Therapeutic Lifestyle Changes (TLC) guidelines developed by the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, 2002).

Randomization to Lifestyle Program
Participants assigned to program 1 (placebo) will be asked to follow the Therapeutic Lifestyle Changes (TLC) diet. Participants will be asked to attend a 90-minute long diet education session, where a Registered Dietitian will explain the TLC guidelines. The first two weeks of the program are referred to as the "lead-in period" (the period of time where all participants follow to the TLC diet). Following the "lead-in period" participants in Program 1 will be asked to continue the TLC diet for 16 weeks. Two meals will be supplied daily for 16 weeks to those participants in the placebo group. Meals will be follow TLC guidelines and will be based on lean-meats for the protein source. The meals will exclude pulses.

Participants assigned to Program 2 will be asked follow the TLC diet and will receive a pulse-based diet. Participants will be asked to attend a 90-minute long education session, where a Registered Dietitian will explain the TLC diet guidelines. Participants will follow the TLC diet during the lead-in period and will be asked to follow the TLC diet for 16 months in addition to consuming a pulse-based diet. The pulse based-diet will include meals prepared with dry peas, lentils, chickpeas, and beans. Two meals will be supplied daily for 16 weeks to those participants on the pulse-based diet program. Meals will contain approximately 90g dried peas, 225 g chickpeas or beans, or 150g lentils. The length of this intervention was based on a review of pulse-based diets for improving insulin and glucose levels (Sievenpiper et al., 2009). Also, the length of intervention is required to observe changes in fertility measures.

Exercise Program
All participants will follow an exercise program for 16 weeks. The program involves 5 sessions of aerobic exercise per week: 3 sessions of supervised exercise training and 2 sessions of unsupervised at-home exercise. The supervised exercise training will occur in the College of Kinesiology at the Williams Building (treadmill walking and exercising on a rowing machine and exercise bike). Each exercise session will be 45 minutes long. This amount of aerobic exercise is the minimal level of exercise shown to be beneficial for increasing high density lipoproteins and lowering triglyceride levels (Durstine, 2002). We also have an exercise training laboratory that is fully equipped with all the required exercise machines and reserved exclusively for research participants. Participants will be

encouraged to exercise at an intensity of at least 60% of their age-predicted maximal heart rate (i.e. 220-age).

Measured Outcomes

PCOS disease measures assessed include: ovarian and uterine morphology (i.e., number and distribution pattern of ovarian follicles, endometrial thickness and pattern as assessed by trans-vaginal ultrasonography) and levels of estradiol, progesterone and an androgen index (a ratio of testosterone to sex hormone binding globulin). These measures are components of routine clinical practice for Drs. Pierson and Chizen.

Metabolic syndrome risk assessment will include fasting plasma glucose, HgA1C, insulin, triglycerides, lipoproteins, and C-reactive protein (for inflammation), as well as measures of abdominal obesity, and blood pressure. All these variables from plasma samples will be determined at our University Hospital or in our research laboratories by trained technicians. Blood pressure, waist girth, and abdominal obesity (by dual energy X-ray absorptiometry) will be measured in our laboratory by trained technicians. All these measurements are routinely performed by Drs. Chilibeck and Zello and have been used by us in previous research studies (Cornish et al., 2009; Little et al., 2009; Vatanparast et al. 2009).

Activity will be measured on three occasions using the Actical, a uniaxial accelerometer that detects vertical acceleration. The Actical (size and dimensions of a small pager) is worn by the participants on a belt worn around the waist with the accelerometer situated at the hip. The women will be instructed both verbally and in written form on how to attach the accelerometer. The participants will be asked to wear the monitor at all times while awake. Exceptions would include water activities like bathing and swimming, or when it is deemed inappropriate by the participant. The participants will be asked to record the time the monitor was attached and removed for the purpose of calculating activity time and sleeping time. The data are electronically downloaded into a data file that contains minute-by-minute movement counts for each participant.

Participants will be asked to complete short questionnaires called the Par-Q Canadian Society for Exercise Physiology, 2002) (Appendix B) and the “Leisure-Time Exercise Questionnaire” to assess physical activity (Godin & Shephard, 1985) (Appendix C). To assess diet, participants will be asked to provide food records (records of what they have eaten for one to four days) (Appendix D). A Pulse Consumption Questionnaire will be administered to determine how often participants eat pulse foods (Appendix E).

Quality of life questionnaires and a brief motivational questionnaire will be administered (Appendix F). (Wallston, Rothman, & Cherrington, 2007; Cronin et al., 1998; Colwell et a., 2010). The questionnaires will be administered via a secured website. Participants will have the option of completing the questionnaires on their home computer or on a study computer in a private area.

We will follow-up with participants at 6 months and 12 months to determine if they are continuing to follow the “standard of care” recommendations (i.e. TLC diet and exercise) and for those randomized to the pulse-based diet if they continue to follow a pulse-based diet and continue to show improvements in measures of metabolic syndrome and PCOS symptoms. With our sample size, we anticipate that we will be able to statistically determine differences in those individuals who continued to follow the pulse-base diet and those who did not.

7. **Time Period (indicate the dates when the research project is expected to begin and to be completed. A final status report must be filed with the Ethics Office once data collection from the last subject is complete. The Ethics Office should be notified once the study site is closed.):**

Timeline			
April 2010 – December 2010	**January 2011-January 2012**	**November 2011 to July 2012**	**January 2012-January 2013**
Ethics submission, hiring research personnel, recruitment and enrollment of participants	TLC-diet lead-in phase. Intervention: 16 weeks per participant (participants phased in to account for seasonal variability)	6 month follow-up	12 month follow-up

8. Data Storage (In accordance with recommended guidelines provide a statement outlining the procedures you will use to store securely the research data. State how long and where the data will be stored and identify the person who will be assuming responsibility for data storage):

Electronic data will be password-protected and stored in a locked office with restricted access (Thorvaldson G30) in the College of Pharmacy and Nutrition for a period of 10 years. Dr. Zello and Ms. Tumback will be responsible for the security of this data.

9. Consent Form (include a copy of the consent form and/or any study information that will be used. If not using a consent form give reasons why).

Attached.

10. Signatures

_______________________________ _______________________________

Printed Name of Principal Investigator Date

________________________________ ________________________________
Signature of Principal Investigator Phone

________________________________ ________________________________
e-mail Fax

________________________________ ________________________________
Signature of Student Researcher(s) Phone

____
e-mail

____
Department Head, Dean, Director, or Administrative Head

11. Contact Person and Mailing Address for Correspondence:

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Appendix C: Adverse Events Forms

PCOS PULSE STUDY

SUBJECT ID #: _______ **SUBJECT INITIALS: _______**

ADVERSE EVENT FORM

Describe the adverse event: ____
____
____
(Record diagnosis where available or describe event in as few words as possible.)

Is this event a **new** event? _____ – assign a new number
Is this event a change/resolution of an **ongoing** event? _____ – use the existing number

Onset of Adverse Event (date): _________________________________
Resolution of Adverse Event (date): ______________________________

Is this event Serious? Yes ☐ No ☐
(Results in death, is life threatening, requires hospitalization, results in persistent or significant disability.*)
Is this event intermittent? Yes ☐ No ☐

1. Rate Intensity (severity):

Mild Moderate Severe Life threatening

2. Is the adverse event is still present: Yes ☐ No ☐

3. Frequency: ____

4. Relationship to experimental procedure (food, exercise or other procedure): Please circle one

Not related Unlikely Possible Probable Definite

Was treatment administered? Yes ☐ No ☐

Details: ____

---

* This is a **SERIOUS ADVERSE EVENT -** will be reported to the Research Ethics Board through their Status Report Form. For definitions of mild, moderate, severe, life threatening, not related, unlikely, possible, probable and definite, refer to MOP (pages 10-16).

Signature:__________________________ Date: ___________________

PI Signature:______________________ Date: _________________

UNIVERSITY OF SASKATCHEWAN

RESEARCH ETHICS BOARD
Ethics Office
Room 302 Kirk Hall, 117 Science Place
Saskatoon, SK S7N 5C8 Canada
Phone: 306-966-2975 Fax: 306-966-2069

INTERNAL SERIOUS ADVERSE EVENT REPORTING FORM

E REPORT PREPARED:	**U OF S REB #:** **10-98**
ICIPAL INVESTIGATOR: **ion Zello/Philip Chilibeck**	**SPONSOR:** **AGRICULTURE AND AGRI-FOOD CANADA**
TOCOL IDENTIFIER / NUMBER:	**DATE RECEIVED** *(Office use only)*:

ORT DATE(S):	**SUBJECT NUMBER:**	**NAME OR MEDICAL TERM OF SAE:**	**TYPE:** initial, f/u, final

SECTION TO BE COMPLETED BY PRINCIPAL INVESTIGATOR:

RELATIONSHIP TO STUDY INTERVENTION			
Unexpected event	**Definitely/Probably Related**	**Possibly Related**	**Unlikely / Unrelated**
☐ Yes ☐ No	☐	☐	☐

TEMENT / SIGNATURE:

Are changes recommended to the protocol, consent form, and/or other? ☐ Yes ☐ No

If yes, please specify: ____

signature attests that the PI has reviewed the SAE and its safety implications, has assessed the relationship to the y intervention of the SAE and attests to the accuracy of the form.

________________________________ ______________________________

ICIPAL INVESTIGATOR DATE

M COMPLETED BY:	**For office use only:**

PLEASE APPEND SUPPORTING DOCUMENTATION (i.e. sponsor SAE report)

Appendix D: Recruitment Poster

UNIVERSITY OF SASKATCHEWAN

Do you have:
Irregular periods?
Difficulty becoming pregnant
Unwanted facial/body hair?
Difficulty losing weight?
A family history of diabetes?

If you have one or more of these symptoms, you may have Polycystic Ovary Syndrome (PCOS).

The Colleges of Pharmacy and Nutrition, Medicine and Kinesiology are conducting a diet and exercise program study for women with PCOS.

Volunteers must be:

- **Between 18 and 38 years old.**
- **Not taking hormonal contraceptives in the last three months.**
- **Not taking fertility medications in the last three months.**

The study runs 20 weeks long, with follow-up at 6 and 12 months.

For more information about participating in this study, please call Dr. Phil Chilibeck at 966-1072 or phil.chilibeck@usask.ca

Appendix E: Inclusion and Exclusion Checklist

INCLUSION AND EXCLUSION CHECKLIST

SUBJECT INITIALS: _______ **RECRUITMENT ID #: _______**

Inclusion Criteria

- Are you a female between the ages of 18 and 38 years — Yes ☐ No ☐
- Have you been diagnosed with PCOS — Yes ☐ No ☐
  Do you have:
  - Irregular periods? — Yes ☐ No ☐
  - Difficulty becoming pregnant? — Yes ☐ No ☐
  - Unwanted facial/body hair? — Yes ☐ No ☐
  - Difficulty losing weight? — Yes ☐ No ☐
  - A family history of diabetes? — Yes ☐ No ☐

Exclusion Criteria

- Have taken hormonal contraceptives in the last three months — Yes ☐ No ☐
- Have taken fertility medications in the last three months — Yes ☐ No ☐
- Do you have any allergies to a pulse-based diet — Yes ☐ No ☐

Additional Information

List any medications (including herbal formulations) you take.

Signature: ________________________________ Date: __________________

Signature of PI: ____________________________ Date: __________________

Appendix F: Medical Assessment Form

Leave space for form

Appendix G: Subject Information Form

Subject Information Sheet

(Use black ink only)

Name:____
(First) (Middle Initial) (Last)

Date of Birth: ____________________ SK Health Card#________________________
(day/month/year)

Address: __________________________ E-mail:_________________________________
____
____
____

Phone: (h) _________________ (w) _________________ (other) _________________

Preferred number and time to call: ____

Physician Name: __________________________ Phone: __________________________

Address:__________________________________
____
____
____
____

Would you like your family physician to be notified of your participation in the study?

- ❑ Yes
- ❑ No

Comments:_________________________________
____
____
____
____
____
____
____

Appendix H: Par-Q

Physical Activity Readiness
Questionnaire - PAR-Q
(revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO	
☐	☐	**1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?**
☐	☐	**2. Do you feel pain in your chest when you do physical activity?**
☐	☐	**3. In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	**4. Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	**5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	**6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	**7. Do you know of <u>any other reason</u> why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to <u>all</u> PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

<u>Informed Use of the PAR-Q</u>: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME ____

SIGNATURE ________________________________ DATE ________________________

SIGNATURE OF PARENT ________________________ WITNESS ________________________
or GUARDIAN (for participants under the age of majority)

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

continued on other side...

Appendix I: Leisure Time Questionnaire

LEISURE-TIME EXERCISE QUESTIONNAIRE

(Godin and Shephard, Canadian Journal of Applied Sport Sciences 10: 141-146, 1985).

* Please do not include activities performed from our study-specific exercise program.

1. Considering a **7-day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your **free-time**?
   (Write the appropriate number in each box)

TIMES PER WEEK

a) STRENUOUS EXERCISE (Heart beats rapidly)
(i.e. running, jogging, hockey, football, soccer, squash, basketball, cross-country skiing, judo, roller skating, vigorous swimming, vigorous long-distance bicycling). ☐

b) MODERATE EXERCISE (Not exhausting)
(i.e. fast walking, baseball, tennis, easy bicycling, volley ball, badminton, easy swimming, alpine skiing, popular and folk dancing). ☐

c) MILD EXERCISE (Minimal effort)
(i.e. yoga, archery, bowling, horseshoes, golf, snow-mobiling, easy walking) ☐

2. Considering a **7-day period** (a week), during your **leisure-time,** how often do you engage in any regular activity long enough to **work up a sweat** (heart beats rapidly)?

OFTEN	**SOMETIMES**	**NEVER/RARELY**
1. ❐	2. ❐	3. ❐

Appendix J: Food Record

Subject code________________________

How to Use the Food Diary

1. It is important to keep up-to date notes in your food diary. List foods immediately after they are eaten. **Please print or write legibly all entries**.

2. Please record only one food item per line in this record booklet.

3. Be as specific as possible when describing the food item that you eat. We need to know the way you cook your food (if food was cooked) and the amount that was eaten.

4. Include the method that was used to prepare the food item - for example: **fresh, frozen, stewed, fried, baked, canned, broiled, raw** or **braised**.

5. Include brand names and restaurant names whenever possible.

6. Report only the food portion that was actually eaten - for example: chicken leg**, 3 oz. baked with skin.** (Do not include the weight of the bones and indicate part of food eaten.)

7. Record amounts as weights or volumes. You may use household measures - for example: **tablespoons, cups, slices** or **units**, as in **one cup skim milk**.

8. For canned foods, include the type of liquid in which it was canned: **sliced peaches in heavy syrup, fruit cocktail in light syrup** or **tuna in water**.

9. Do not alter your normal diet habits during the time that you keep this diary.

10. Remember to record the amounts of fats used in mixed dishes (such as sandwiches). These fats include oils, butter, salad dressings, margarine, miracle whip, mayonaise.

PLEASE LIST Every FOOD and DRINK as you consume it **DATE: ___________________________**

Time	Food Items	Type & Preparation	Amount	Brand Name or Where Bought	Code
Morning					
Mid-morning					
Noon Meal					
Midday					
Evening Meal					
Before Bed					

Was this intake usual? Circle one: Yes No (if No, explain why not ____________________________________)

Did you take any vitamins/minerals during this time? Circle one: Yes No (if Yes, list names on next page)

BRAND NAME OF SUPPLEMENT	NUTRIENTS	AMOUNT

Recipe Information:
____
____
____
____
____
____
____

Appendix K: Accelerometer Log

Activity Monitor Log Sheet

- ___ ___ ___ **SERIAL NUMBER: C 8 4_____________**

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	
Date									
On at wake up (time you put monitor on for the day)									Please return this log and your accelerometer to the TLC guidelines presentation
Off at bedtime (time you took off monitor before bed)									

NOTES
1. The activity monitor is to be worn securely on the RIGHT hip using the belt provided.
2. The monitor can be worn over or under your clothing (your choice).
3. Although the monitors are fairly durable, care should be taken when wearing them as they are quite expensive.
4. Please wear your monitor everyday, even if you've stayed home from work (e.g., due to illness).

NOTE: If you have to take off the monitor for any reason please record the reason why below.

Appendix L: Aerobic Exercise Tracking Logs

Tracking Log for Supervised Aerobic Exercise

Name: **Age:**
Aerobic Training
Heart Rate Ranges: 60 – 75% of Max Heart Rate
_______Beats per 10sec. count

Date:	Type of Training	Duration	Intensity	
			HR 1	HR 2

Tracking Log for Unsupervised Aerobic Exercise

Funded by:

Agriculture and Agri-Food Canada

Pulse Growers of Saskatchewan

University of Saskatchewan
966-1082

College of Kinesiology
Phil Chilibeck Ph.D.
87 Campus Drive
Saskatoon, SK S7N 5B2

e-mail:phil.chilibeck@usask.ca

A Lifestyle Intervention for Women with Polycystic Ovary Syndrome: The Role of a Pulse-Based Diet and Aerobic Exercise on Infertility Measures and Metabolic Syndrome Risk

Aerobic Exercising at home Tracking Log Book

Write the type of Aerobic exercise and duration of exercise in appropriate box. Check off HR 1 and HR 2 after reading your Heart Rate. Write date as shown below. Please contact 966-1305 or 966-1082 if you have any questions or concerns.

Aerobic Training
Heart Rate Ranges: 60 – 75% of Max Heart Rate
_______Beats per 10sec. count

Date:	Type	Duration	Intensity	
			HR 1	HR 2
Dec.	Bike	35min	√	√

17/08				

Date:	Type	Duration	Intensity			Date:	Type	Duration	Intensity	
			HR 1	HR 2					HR 1	HR 2

Name:_________________________

Appendix M: Online Questionnaire Instructions

Leave room for instructions

Study Log 1

Phase 1: Lead-In (weeks 1-2)

Participant Code:________

UNIVERSITY OF SASKATCHEWAN

Please write on the back of this page if there is not enough room for your responses	
WEEK 1 DAY 1	**Date:**
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
WEEK 1 DAY 2	**Date:**
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
WEEK 1 DAY 3	**Date:**
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
WEEK 1 DAY 4	**Date:**
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
Please write on the back of this page if there is not enough room for your responses	
WEEK 1 DAY 5	**Date:**
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
WEEK 1 DAY 6	
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
WEEK 1 DAY 7	
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
Have there been changes in the medications or supplements you are taking? If yes, please list:	

Please write on the back of this page if there is not enough room for your responses	
WEEK 2 DAY 1	**Date:**
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you eat differently from the guidelines.	
WEEK 2 DAY 2	**Date:**
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
WEEK 2 DAY 3	**Date:**
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
WEEK 2 DAY 4	**Date:**
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
Please write on the back of this page if there is not enough room for your responses	
WEEK 2 DAY 5	**Date:**
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
WEEK 2 DAY 6	
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
WEEK 2 DAY 7	
TLC DIET GUIDELINES	
Did you follow the TLC Guidelines today?	Yes No
If no, please state what you ate that was different from the guidelines.	
Have there been changes in the medications or Supplements you are taking? If yes, please list:	

Appendix O: Study Log 2

Study Log 2

Phase 2: Program (weeks 3-18)

Participant Code:________

Please write on the back of this page if there is not enough room for your responses.				
WEEK __________	**Day 1**	**Day 2**	**Day 3**	**Day 4**
MEAL 1				
Recipe name of meal 1				
Percent eaten (out of 100%)				
If not 100% eaten, please tell us why not				
MEAL 2				
Recipe name of meal 2				
Percent eaten (out of 100%)				
If not 100% eaten, please tell us why not				
TLC DIET GUIDELINES				
Did you follow the TLC Guidelines for breakfast and snacks today?	Yes No	Yes No	Yes No	Yes No
If no, please state what you ate that was different from the guidelines.				
EXERCISE				
Did you exercise today? (circle one)	Yes No	Yes No	Yes No	Yes No
What kind of exercise? How long?				
OTHER COMMENTS				
Is today different than usual days (for example are you sick, away from home...)?				

Please write on the back of this page if there is not enough room for your responses.			
WEEK ____________	**Day 5**	**Day 6**	**Day 7**
MEAL 1			
Recipe name of meal 1			
Percent eaten (out of 100%)			
If not 100% eaten, please tell us why not			
MEAL 2			
Recipe name of meal 2			
Percent eaten (out of 100%)			
If not 100% eaten, please tell us why not			
TLC DIET GUIDELINES			
Did you follow the TLC Guidelines for breakfast and snacks today?	Yes No	Yes No	Yes No
If no, please state what you ate that was different from the guidelines.			
EXERCISE			
Did you exercise today? (circle one)	Yes No	Yes No	Yes No
What kind of exercise? How long?			
OTHER COMMENTS			
Is today different than usual days (for example are you sick, away from home...)?			

Have there been changes in the medications or supplements you are taking? If yes, please list changes:

Appendix P: Physical Measures Data Collection Form

PHYSICAL MEASURES DATA COLLECTION FORM
PCOS STUDY

Participant Code	
Date	
Measure **Check one**	☐ Week 1 (Enrolment) ☐ Week 4 (End of lead-in period) ☐ Week 20 (End of 16-week program) ☐ 6-Month Follow-Up ☐ 12-Month Follow-Up
Blood Pressure	
Weight	
Height	
Waist Circumference	

REVISED PROTOCOL FOR MEASUREMENT OF WAIST CIRCUMFERENCE

The article by McGuire and Ross describes an important advancement in current international waist circumference measurement guidelines. As outlined by the authors, compelling literature indicates that the specific waist circumference protocol does not change the well-established relationships between waist circumference and the risk of premature mortality and chronic disease (such as cardiovascular disease and diabetes). These relationships appear to be consistent across sex, race and ethnicity.

This article has significant implications for CSEP-certified health and fitness practitioners. The authors advocate the use of the National Institutes of Health method to measure waist circumference rather than the World Health Organization (WHO) measurement currently employed in the CPAFLA (i.e., the midpoint between the lower border of the rib cage and the iliac crest). The NIH method consists of measuring waist circumference at the superior border of the iliac crest. It is anticipated that the use of a bony landmark will improve the reliability of waist circumference measurements during self-assessments and appraisals by CSEP health and fitness practitioners.

The CSEP Health and Fitness Program has officially accepted the recommendations of Dr. Ross and colleagues in an attempt to standardize the measurement of waist circumference. Therefore, effective immediately all CSEP-certified health and fitness practitioners are recommended to use the NIH method to assess waist circumference (as outlined in the article by McGuire and Ross). ***It is important to note that health and fitness practitioners will be able to continue to use the current body composition tables in the CPAFLA****. It is anticipated that the differences between assessment techniques are within the range of error of measurement. More importantly, the measurement site does not appear to affect the relationship with the risk for chronic disease and premature mortality.*

It is hoped that these actions will improve the ease of administration and reliability of the waist circumference measurement in the CPAFLA appraisal. In a subsequent revision of the CPAFLA, the CSEP Health & Fitness Program will update the waist circumference measurement section to reflect this important change.

Appendix Q: Revised Protocol for Measurement of Waist Circumference

■ BACKGROUND

Waist circumference (WC) is commonly used to assess abdominal obesity and has been established as a predictor of increased morbidity and mortality independent of body mass index[1]. Individuals with increased WC values are more likely to have hypertension, type 2 diabetes, dislipidemia, and the metabolic syndrome than individuals with normal WC values regardless of weight status[2]. In addition, WC predicts the development of diabetes beyond that explained by commonly evaluated cardiometabolic risk factors including blood pressure, lipoproteins, glucose levels, and body mass index[3].

Despite the literature establishing WC as an independent predictor of morbidity and mortality, there is currently no consensus on the optimal protocol for measurement of WC; furthermore there is no scientific rationale for any of the protocols currently recommended by leading health authorities. Common measurement sites are the visible narrowing of the waist, the last rib, top of the iliac crest or the midpoint between the last rib and iliac crest. Recently, a panel of experts convened to evaluate the influence of the measurement protocol on the relationships between WC with morbidity from cardiovascular disease and type 2 diabetes, and with mortality from all causes and from cardiovascular disease[4]. The findings indicated that WC protocol had no substantial influence on the relationships between WC and morbidity of cardiovascular disease and diabetes and all-cause mortality and cardiovascular disease mortality. Moreover, similar associations were observed across sex, race, and ethnicity.

In the absence of a clear biological rationale, the panel recommended the protocol that was the most practical and would facilitate adoption by both the general public and the practitioner. The protocol of choice was required to have two fundamental features: 1) the use of a bony landmark and, 2) ease of measurement. These features would help to ensure reliable measures, promote adoption by both practitioner and lay public, and facilitate training and instruction. It was recognized that the protocols of the National Institutes of Health (NIH) (superior border of the iliac crest) and the World Health Organization (WHO) (midpoint between the lower border of the rib cage and the iliac crest) are both based on the use of bony landmarks to identify the proper WC location. However, the panel's consensus opinion was that the general public would be more likely to adopt the NIH protocol as it requires only a single palpation of the iliac crest whereas the WHO protocol requires the measurement of distance and calculation of the midpoint between two bony landmarks. Thus, the NIH protocol might be more feasible for self-measurement.

In support of the recommendations from the expert panel, the *2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children*[5] recently suggested that practitioners utilize the NIH method to obtain a measurement of WC. In an attempt to standardize the measurement protocol and avoid confusion between practitioners and the general public alike, CSEP has now adopted the NIH measurement protocol.

■ MEASUREMENT OF WAIST CIRCUMFERENCE

Equipment

K-E Anthropometric tape or equivalent

Procedure

Clear the client's abdomen of all clothing and accessories. Position the client with feet shoulder width apart and arms crossed over the chest in a relaxed manner. Take a position to the right side of the client's body on one knee.

Using the NIH protocol, the waist circumference measurement should be taken at the top of the iliac crest. To find this landmark, palpate the upper right hipbone of the client until you locate the uppermost lateral border of the iliac crest. Draw a horizontal line at this landmark at the midline of the body.

Position the tape directly around the abdomen so that the inferior edge of the tape is at the level of the landmarked point. Use a cross-handed technique to bring the zero line of the tape in line with the measuring aspect of the tape. Ensure that the measuring tape is positioned in a horizontal plane around the abdomen. Apply tension to the tape to ensure it is snug, without causing indentation to the skin. At the end of a normal expiration, take the measurement to the nearest 0.5cm.

■ REFERENCES

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This article was written by K. Ashlee McGuire and Robert Ross, Queen's University, Kingston, Ontario, for the Canadian Society for Exercise Physiology and it is endorsed by the CSEP Knowledge Translation Committee. Introduction courtesy of Dr. Darren E.R. Warburton, University of British Columbia, Vancouver BC.

This document is also located in the *Knowledge Translation* section of the CSEP website, www.csep.ca/forms

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Appendix R: Fitness Testing Data Form

SUBJECT INITIALS: _______ **RECRUITMENT ID #:_________**

TREATMENT: Fitness Testing – Baseline

Date of measurement: ______________________
(Day/month/year)

Was DXA scan completed? Yes ☐ No ☐

Vital Signs

BP: ________________ (mmHg)
________________ (mmHg)
________________ (mmHg)
HR: ________________ (bpm)

Anthropometry

Waist Girth: ________________ (mm)
Weight: ________________ (kg)
Height: ________________ (m)
BMI: ________________

Form Complete by: ____________________ Date: _________________________

Signature of PI: ________________________ Date: _________________________